UNIVERSITEIT GENT

FACULTY OF SCIENCES
Department of Organic and Macromolecular Chemistry
Polymer Chemistry Research Group

Triazolinediones for the development of a versatile click chemistry platform applied in polymer science

Stijn BILLIET

Promotor: Prof. Dr. Filip Du Prez
Co-promotor: Prof. Dr. Johan Winne

Ghent, 2016

Thesis submitted to obtain the degree of Doctor of Science: Chemistry

Exam Commission

Prof. Dr. Annemieke Madder (Ghent University)

Prof. Dr. Bruno De Geest (Ghent University)

Prof. Dr. David Fournier (Ghent University & Université de Lille)

Prof. Dr. Helmut Schlaad (University of Potsdam)

Prof. Dr. Thomas Junkers (Hasselt University)

Dr. Laetitia Mespouille (University of Mons)

Prof. Dr. Johan Winne (Ghent University)

Prof. Dr. Filip Du Prez (Ghent University)

Research funded by the Agency for Innovation by Science and Technology in Flanders

Onderzoek gefinancieerd door het Agentschap voor Innovatie
door Wetenschap en Technologie

The meeting of two personalities is like the contact of two chemical substances: if there is any reaction, both are transformed.

C. G. Jung

Acknowledgements

The publication of this PhD manuscript is the result of four years of work in the laboratory. As with any project, performing research is a learning process and I was blessed with the advise and support of remarkable people. Without their support and guidance, I would not have been able to finish this adventure, therefore I want to take the time to properly thank those people.

First of all I would like to express my special thanks and appreciation to my promotor professor dr. Filip E. Du Prez, for the opportunity to perform my PhD in his research group. His door was always open for me to drop by (*yet another time*) when I needed his advice. The friendly atmosphere he created made me feel at home from the first day and is an important reason why I could finish this thesis. Filip, thank you for all the chances you gave me to develop myself as a researcher and as a group leader. I highly appreciate all the times you involved me in (industrial) projects and valued my opinion in these. Thanks to your trust and support, I was able to acquire unique experiences that changed me as a person and that will definitely help me in the rest of my career.

At the same level, I would like to thank my co-promotor professor dr. Johan Winne. I cannot count the numerous times I could trouble you during these last years with yet another NMR or a failed synthesis. You always took the time to explain everything in full detail and made sure I left your office with something new to think about. You have been a tremendous mentor for me and it has been an enormous honour to work with a true genius as yourself. I would like to thank you for encouraging my research and for allowing me to grow as a chemist.

Next, I would like to thank dr. Bart Hommez for his important role in all industrial projects that emerged out of this doctoral work. Our frequent discussions and conversations regarding future projects and perspectives were extremely valuable to me and helped me

to get a better understanding of what the chemical industry is about. Thanks to your advise, helpful *tips and tricks* and guidance I feel more confident to take the next steps in my career.

I also would like to acknowledge the group of professor José Martins and especially Tim Courtin for the NMR measurements, Jan Goeman for the MS analyses and Joke Lenoir for the toxicity tests. A special thanks as well to Tom, Carine, Christel and Queenie for all their help concerning administration regarding the last four years.

Of course I cannot forget all the members of the PCR group who created a unique atmosphere to work in. I would especially would like to thank some people that made my life in the lab a lot more easy and enjoyable:

- Bernhard, without you doing research in our lab would be so much more difficult. Thanks for your countless efforts to make everything easier in and around the lab. I will always remember our road trips and the laughs we shared.

- Kevin, I don't think I can describe in a few sentences how important your part was in my phD. During the first days of your master thesis, I realized there were as many moments (or even more) where you taught me new things than the other way around. Also thanks for all the times you have spent helping with figures, graphs and other computer stuff, this work wouldn't have been the same without you.

- Hannes for all your hard work, making your deadlines (not mine but yours), the beers, jokes and so much more.

- Xander, for all the laughs and beers we shared. But also for co-founding our (now yearly) team event, the ´komen drinken' and the weekly happy hours. Being at the beginning of these 'traditions' is one of the things I will treasure the most.

- Bastiaan for all the help in bringing this chemistry to the next level. Your help is truly appreciated and made it possible for me to focus on other projects which would not have been possible without you.

- Lae & Stef for the laughs in and around the lab.

A special thanks as well to professor Christopher Barner-Kowollik who was so kind to invite me to work for two months in his lab in Karlsruhe, Germany. It was an eye-opening experience and I enjoyed every last minute of it. A lot of this can be related to the wonderful people (Jan, Kim, Anja, Oczan, Astrid, Michi...) I was fortunate to work with (and discover Karslruhe).

Of course I cannot forget professor Craig Hawker, thanks to whom I have spent the last five months of my research in Santa Barbara, California. It was a true honour working for a world-known scientist as yourself. Everyday I learned new insights and skills that will stay with me for the rest of my life. Also a big thanks to Will, Kaila, Christian and Anna to make me feel at home from the beginning and helping me at every turn.

I would like to thank my parents, brother, sister and family(in-law) for always being supportive and taking an interest in what I was doing. It was not always easy to explain what I was working on, but nevertheless thanks to your support and your listening ear, you made it all a bit easier. Also a big thanks to my friends, to take mind of work and made these last four years so enjoyable.

And last but not least, I would like to take the opportunity to thank the most important person in my life: Lien. Since a few days ago, I can proudly call you my wife. Thanks to your endless support, bad moments disappeared with a single smile while sharing the good moments made it even more special. You are the biggest reason that I was able to finish this thesis and I will love you until the end of time.

Stijn

Table of Contents

Used abbreviations

AAC	Azide-alkyne cycloaddition
AE	Alder-ene
AFM	Atom force microscopy
AI	Aziridinium
AIBN	Azobisisobutyronitrile
Arg	Arginine
ATRP	Atom transfer radical polymerization
bisTAD	Bifunctional TAD molecule
br.	Broad
BSA	Bovine serum albumin
BuPAT	2-([Butylsulfanyl-carbonothioyl]sulfanyl)propanoic acid
BuTAD	4-Butyl-1,2,4-triazoline-3,5-dione
BUr	4-Butyl-1,2,4-triazolidine-3,5-dione
Cbz	Carboxybenzyl
CCl_4	Carbon tetrachloride
$CDCl_3$	Chloroform-d
CMM	Centre of molecular modeling
CO	Carbon monoxide
Cp	Cyclopentadiene
CRP	Controlled radical polymerization
CTA	Chain transfer agent

Cu	Copper
CuAAC	Copper(I) catalyzed azide-alkyne cycloaddition
d	doublet
$Đ$	Dispersity
DA	Diels-Alder
DABCO	1,4-Diazabicyclo[2.2.2]octane
DABCO-Br	tetramer 1,4-Diazabicyclo[2.2.2]octanebromide complex
DBU	1.8 Diazobicyclo(5.4.0)undec-7-ene
DCC	N,N'-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DFT	Density functional theory
DiPEA	N,N-Diisopropylethylamine
DMA	Dynamic mechanical analysis
DMAP	4-Dimethylaminopyridine
DMB	2,3-Dimethyl-2-butene
DMEQ	3-Carboxy-6,7-demethoxy-1-methyl-2(1H)-quinoxalinone
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
DMSO	Dimethylsulfoxide
DMSO-d_6	Dimethylsulfoxide-d_6
DPPA	Diphenyl phosphoryl azide
DSC	Differential scanning calorimetry
d	Doublet
EAS	Electrophilic aromatic substitution
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDG	Electron donating group
ELSD	Evaporative light-scattering detector
ESI	Electron spray ionization

EWG	Electron withdrawing group
FMP	2-Formyl-3-methylphenonoxy
FRET	Fluorescence resonance energy transfer
FT-IR	Fourier-transform infrared
GC	Gas chromatography
GC-MS	Gas chromatography mass spectrometry
Glu	Glutamic acid
HDA	Hetero-Diels-Alder
HDD	2,4-Hexadiene-1,6-diol
HDEO	*trans*,*trans*-2,4-Hexadiene-1-ol
HDI	Hexamethylene diisocyanate
His	Histidine
HIV	Human immunodeficiency virus
HOMO	Highest occupied molecular orbital
HPLC	High performance liquid chromatography
HSA	Human serum albumin
IPDI	Isophorone diisocyanate
IPDI-TAD	Isophorone bis-1,2,4-triazoline-3,5-dione
IR	Infrared
IRC	Intrinsic reaction coordinate
IZ	Iminium–urazolide zwitterionic intermediate
k_2	Rate constant
LAH	Lithium aluminium hydride
LC-MS	Liquid chromatography - mass spectrometry

LC-SEC	Liquid chromatography - size exclusion chromatography
LUMO	Lower occupied molecular orbital
Lys	Lysine
m	Multiplet
M	Molar
MALDI	Matrix-assisted laser desorption ionization
MDI	4,4'-Methylenebis(phenyl isocyanate)
MDI-TAD	4,4'-(4,4'-Diphenylmethylene)-bis-(1,2,4-triazoline-3,5-dione)
MeTAD	4-Methyl-1,2,4-triazoline-3,5-dione
Me_6TREN	Tris[2-(dimethylamino)ethyl]amine
min	Minutes
M_n	Numeric average molecular weight
MO	Molecular orbital
MRFA	Methionine-arginine-phenylalanine-alanine
MS	Mass spectrometry
M_w	Weight average molecular weight
N	Normality
NHS	N-Hydroxysuccinimid
NMR	Nuclear magnetic resonance
OZ	Open zwitterion
P	Product
PBA	Polybutylacrylate
PCM	Polarizable continuum model
PCR group	Polymer Chemistry Research group
PD	Polarized diradical

Pd/C	Palladium on carbon
PiBA	Poly(isobornylacrylate)
PhD	Doctor of philosophy
PhTAD	4-Phenyl-1,2,4-triazoline-3,5-dione
PhUr	4-Phenyl-1,2,4-triazolidine-3,5-dione
PDMAS	Poly(4-(N,N-dimethylamino)styrene)
PDMS	Poly(dimethylsiloxane)
PEG	Poly(ethyleneglycol)
PFMA	Poly(furfuryl methacrylate)
PMMA	Poly(methyl methacrylate)
PMDETA	N,N,N',N",N"-Pentamethyldiethylenetriamine
PMVP	Poly(N-methyl-2-vinyl-pyrrole)
PPE	Poly(oxy-2,6-dimethyl-1,4-phenylene)
PPO	Polypropyleneoxide
ppm	Parts per million
PRC	Pre-reactive complex
PS	Polystyrene
PTFE	Polytetrafluoroethylene
PTMS	Poly(2,4,6-trimethoxystyrene)
PVC	Polyvinylchloride
q	Quadruplet
quin	Quintuplet
rAE	Retro-Alder-*ene*
RAFT	Radical addition-fragmentation transfer
RCS	Refrigerated cooling system
rDA	Retro-Diels-Alder
ref	Reference
r_f	Retardation factor

RNA	Ribonucleic acid
ROMP	Ring-opening metathesis polymerisation
s	Singlet
SAXS	Small-angle X-ray scattering
Ser	Serine
SEC	Size exclusion chromatography
sec	Seconds
sext	Sextuplet
SM	Starting material
SMI	Slug mucosal irritation
t	Triplet
TAD	1,2,4-Triazoline-3,5-dione
TEA	Triethylamine
T_{deg}	Degradation temperature
T_g	Glas transition temperature
TGA	Thermogravimetric analysis
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMEDA	N,N,N',N'-Tetramethylethylenediamine
TMS	Trimethoxystyrene
Trp	Tryptophan
TS	Transition State
t	Triplet
UGent	Ghent University
UV	Ultraviolet
VIS	Visible

w%	Weight percentage
XPS	X-ray photoelectron spectroscopy

Chapter I

General aim and outline

I.1 General background and aim

I.1.1 *Click* chemistry

In 2001, Kolb, Finn and Sharpless created a paradigm shift by publishing a review describing a *new* strategy for organic chemistry, which they named *click* chemistry. The name *click* chemistry actually describes a 'guiding principle', which meets the demands of modern day research applications and in the case of the authors, the field of drug discovery.[1] However, this concept was quickly taken up by other fields, for example polymer science. While nature possesses the remarkable ability to perfectly control reversible carbonyl chemistry to make new covalent bonds, synthetic chemists are not able to accomplish this. Instead the focus (of *click* chemistry) shifts to highly energetic 'spring loaded' reactants - reliable and selective processes controlled by kinetics. Sharpless and co-workers proposed that reactions must meet a set of strict criteria - *'modular, wide in scope, high yielding, create only inoffensive by-products (that can be removed without chromatography), be stereospecific, simple to perform and that require benign or easily removed solvents'* - to be classified as a *click* reaction.[2] Additionally, in the ideal case, the used starting materials and reagents should be readily available. Meeting all requirements to be classified as a *click* reaction is not trivial, however several reactions have been identified in this regard, the most well-known being the *copper(I)-catalyzed azide-alkyne cycloaddition* (CuAAC).[3,4] Slowly more chemistries took a claim on the title of *click* reaction, such as nucleophilic ring opening reactions (epoxides, aziridines, aziridinium ions), non-aldol car-

bonyl chemistry (formation of ureas, oximes and hydrazones), additions to carbon–carbon multiple bonds and cycloaddition reactions. When the (limited amount of) industrial projects that were started involving this chemistry (Invitrogen™, Allozyne™, Aileron™, Integrated Diagnostics™, Baseclick™, Active Motif Chromeon™, Cyandye™...) are looked at in more detail, it becomes apparent that *click* chemistry finds most of its applications in two main areas: the life sciences and materials science. An example of the former is the addition of a labelling group to a biomolecule, while in the case of the materials science this doctoral work tries to serve as an example.

I.1.2 *Click* chemistry in polymer science

Although Sharpless and co-workers envisaged the main applications of *click* chemistry in the field of biologically active molecules, the impact this work has made on polymer science might be equally significant.[5] Indeed, when designing and synthesising functionalized polymer architectures, the efficient *click* reactions can play a huge role - this in combination with the lack of side products and therefore easier purification. This has even led to the synthesis of polymer materials that were not achievable otherwise.[6] With the success of *click* chemistry (and everything concerning it), the number of publications increased immensely, leading in some cases to the misuse of the term. To avoid further confusion, an expansion of the core principle of *click* chemistry for use in polymer science was introduced.[7] Figure I.1 shows the originally defined characteristics in combination with the newly introduced 'polymer principles'. Two main extra features, large scale purification and equimolarity, were introduced in this way. The 'traditional' purification methods (e.g. distillation) are not possible when working with polymers; additionally the large scale at which most polymers are synthesized complicates this even more. The other criterion, equimolarity, might be the most crucial one, as a *clean* polymer-polymer ligation is not possible without it.[8–10]

Since the introduction of the 'expanded *click* definition', the search for suitable combinations of modular reagents has continued in the field of polymer synthesis.[11,12] However, the quest for a truly versatile system still continues. In the ideal case, such a system, besides having all the characteristics associated with the *click* concept, should also offer a choice between irreversible and reversible connections. This might be counter-intuitive to

the *click* concept which favours robust thermodynamically driven reactions, but especially in the field of polymer materials dynamic or reversible covalent bonds are highly desired (e.g. for self-healing, recycling or network malleability). Up to this point, no general *click* chemistry system has been reported that can be tuned for either reversible or completely irreversible covalent linking within useful temperature ranges.

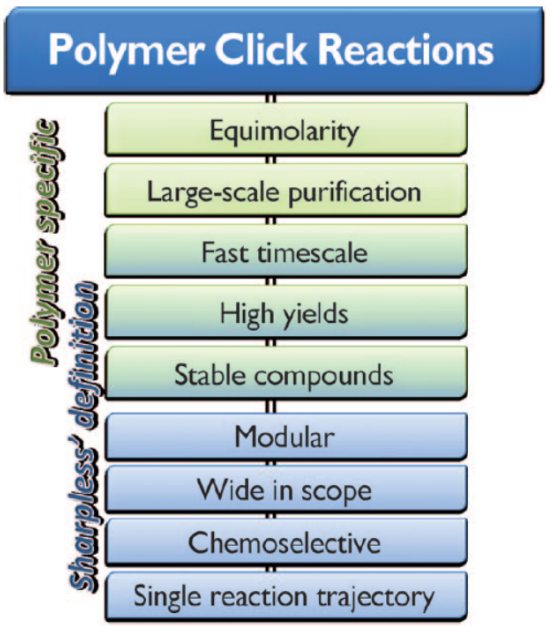


Figure I.1: Requirements for click reactions involving one or more polymeric reagents (blue: originally defined by Sharpless; green and blue–green: adapted requirement related to synthetic polymer chemistry). Reprinted with permission - Copyright (2011) Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.[7]

I.1.3 Triazolinedione-based *click* chemistry

1,2,4-Triazoline-3,5-dione (TAD) molecules are heterocyclic compounds with an azo-moiety connected to two carbonyl functionalities.[13] These strained diazodicarboxylate derivatives show a remarkable reactivity that can be compared to singlet oxygen, thus favouring *ultrafast* Diels-Alder and Alder-*ene* reactions (see Figure I.2).

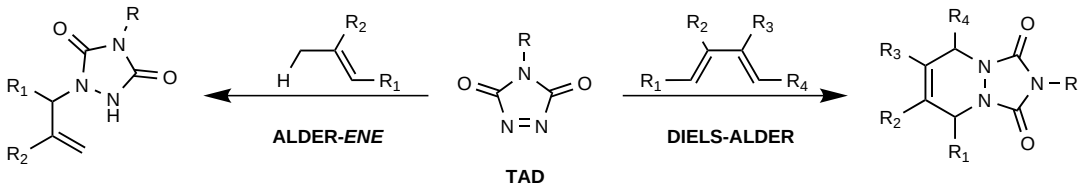


Figure I.2: A triazolinedione (TAD) molecule can undergo a Diels-Alder (right) or an Alder-*ene* reaction (left).

Diels-Alder and Alder-*ene* reactions are most known for their orthogonality and reversibility - although most systems (such as the well-known Diels–Alder reaction of furans and maleimides) do not meet all *click* chemistry requirements.[14–16] Conversely, TAD compounds offer a range of selective and predictable covalent linking reactions that are high yielding under equimolar conditions at low temperature (<20 °C), without the need for a catalyst.[17] An additional benefit of these reactions is the intense red colour of TAD compounds, which provides a visual feedback system, as most of the corresponding reaction products are colourless.[18]

Although some work has already investigated the use of TAD moieties in *click-like* reactions[19], no extensive study has been devoted to the *click* properties of Diels-Alder and Alder-*ene* type reactions.

I.1.4 General aims of this doctoral work

In this doctoral research, the general aim consisted in using triazolinediones as tool for the development of a versatile *click* chemistry platform. We aimed to provide the user the choice of tuning the kinetic and the thermodynamic behaviour. At the start of this PhD research, no such system that covers all these aspects had been reported. Moreover, examples of the use of triazolinediones in both Alder-*ene* and Diels-Alder context for the construction of polymer-based materials will be reported.

I.2 Outline

Chapter II gives an overview of the theoretical background of triazolinediones (TADs). In a first step, focus is placed on the synthesis of these components. The synthetic part is divided in synthesis of urazoles (the precursor of triazolinediones) and the oxidation of urazoles to yield TADs. This is followed by a short overview of the remarkable reactivity of TAD molecules, the focal point being the reactions that play an important role in this work. After this an overview is provided of the use of triazolinediones in polymer science. In a last part TAD molecules are described in the context of *click-like* chemistry.

Chapter III describes all the relevant properties of triazolinediones. The detailed discussion of relevant features (orthogonality, kinetics, introduction of functional groups,

stability and toxicity) is also supported by experiments on the small molecule-level and is crucial to understand TAD chemistry and be able to use it in an efficient way on the polymer level.

Chapter IV will provide, in a detailed way, a check for the possible *click* behaviour of TAD chemistry and more specifically in a Diels-Alder and Alder-*ene* context. To achieve this, model studies on low molecular weight components were conducted and supported by theoretical rationalizations. In a next step, examples of these TAD-based *click* reactions on macromolecular level are discussed.

Chapter V describes the additive-free preparation of polymer materials by combining TAD chemistry with readily available commercial plant oils. In a first step, model studies on the most common natural fatty acids are described which establish the main modes of reactivity of TAD with various natural fatty acid side chains. Then, a series of synthesized, bifunctional TAD molecules was used for the chemical crosslinking of crude plant oils to obtain a large variety of polymer networks with a wide range of properties.

Chapter VI describes the reversible *click* chemistry platform that was developed using the unique reactivity of triazolinediones with indoles. These molecules can undergo a *click* reaction but when brought to elevated temperature the formed adducts show reversibility. In this chapter the new concept of '*transclick*' reaction is introduced, showing a *controlled sequence of click reactions*. This is first thoroughly studied on low molecular weight model studies, followed by selected examples on polymer level.

Chapter VII adds an extra level to the newly introduced reversible *click* chemistry platform. By altering the chemical substitution of the used indole components, a lower reversibility temperature can be achieved. This provided the '*transclick*' platform with one extra 'step' and thus expanding the *controlled sequence of click reactions*. Similar strategies as the previous chapter were followed to demonstrate this concept on low molecular weight components. Unfortunately due to time constraints it was not possible to include data describing the new level of *transclick* on polymer level in this doctoral work.

Chapter VIII will give a general conclusion of the described work and address some possible future directions for the newly developed *click* chemistry platform.

Chapter IX provides a summary of this doctoral work in Dutch.

I.3 Bibliography

[1] J. E. Moses, A. D. Moorhouse, *Chemical Society Reviews* **2007**, *36*, 1249–1262.

[2] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angewandte Chemie International Edition* **2001**, *40*, 2004–2021.

[3] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angewandte Chemie International Edition* **2002**, *114*, 2708–2711.

[4] C. W. Tornøe, C. Christensen, M. Meldal, *The Journal of Organic Chemistry* **2002**, *67*, 3057–3064.

[5] C. J. Hawker, V. V. Fokin, M. G. Finn, K. B. Sharpless, *Australian Journal of Chemistry* **2007**, *60*, 381–383.

[6] A. Inglis, S. Sinnwell, M. Stenzel, C. Barner-Kowollik, *Angewandte Chemie International Edition* **2009**, *48*, 2411–2414.

[7] C. Barner-Kowollik, F. E. Du Prez, P. Espeel, C. J. Hawker, T. Junkers, H. Schlaad, W. Van Camp, *Angewandte Chemie International Edition* **2011**, *50*, 60–62.

[8] W. Xi, T. F. Scott, C. J. Kloxin, C. N. Bowman, *Advanced Functional Materials* **2014**, *24*, 2572–2590.

[9] B. S. Sumerlin, A. P. Vogt, *Macromolecules* **2010**, *43*, 1–13.

[10] P. L. Golas, K. Matyjaszewski, *Chemical Society Reviews* **2010**, *39*, 1338–1354.

[11] T. Pauloehrl, G. Delaittre, V. Winkler, A. Welle, M. Bruns, H. G. Börner, A. M. Greiner, M. Bastmeyer, C. Barner-Kowollik, *Angewandte Chemie International Edition* **2012**, *51*, 1071–1074.

[12] M. A. Tasdelen, Y. Yagci, *Angewandte Chemie International Edition* **2013**, *52*, 5930–5938.

[13] R. C. Cookson, S. S. H. Gilani, I. D. R. Stevens, *Tetrahedron Letters* **1962**, *3*, 615–618.

[14] M. A. Tasdelen, *Polymer Chemistry* **2011**, *2*, 2133–2145.

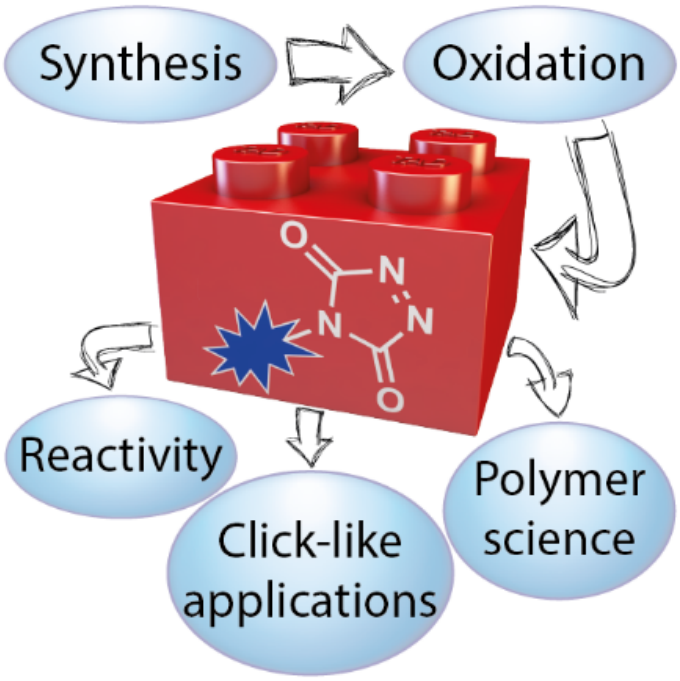
[15] J.-A. Funel, S. Abele, *Angewandte Chemie International Edition* **2013**, *52*, 3822–3863.

[16] K. C. Nicolaou, S. A. Snyder, T. Montagnon, G. Vassilikogiannakis, *Angewandte Chemie International Edition* **2002**, *41*, 1668–1698.

[17] S. Radl in *1,2,4-Triazoline-3,5-Diones*, *Vol. 67*, Elsevier, San Diego, **1997**.

[18] I. K. Korobitsyna, A. V. Khalikova, L. L. Rodina, N. P. Shusherina, *Chemistry of Heterocyclic Compounds* **1983**, *19*, 117–136.

[19] C. Barbas, H. Ban, J. Gavrilyuk, *US20120289682* **2010**, Tyrosine Bioconjugation through Aqueous Ene–Like Reactions.



Abstract:

This chapter gives a theoretical background of triazolinediones in polymer science. Several topics of importance for this doctoral work are highlighted. In a first part the relevant literature concerning the synthesis of urazoles (the precursor molecule of triazolinediones) is discussed. This is followed by a short overview of the available oxidation methods to yield the desired TAD molecules. After this the remarkable reactivity of TAD molecules will be described, while the last parts of this chapter will discuss two case studies, providing insights in the use of triazolinediones in respective macromolecular and *click-like* applications.

Reference:

De Bruycker K., Billiet S., Houck H.A., Chattopadhyay S., Winne J.M., Du Prez F.E., *Chemical Reviews* **2015**, *submitted*.

Chapter II

Introduction to the synthesis, reactivity and applications of triazolinediones

II.1 Introduction

Azo-compounds, with the general structure R−N=N−R', are di-imides that are already used for a very long time in the chemical industry as colourants (in paint, fabric and plastics in general[1,2]) or as a radical source (initiator for radical polymerization[3]). Depending on the stabilization degree of the nitrogen double bond (due to the substituents R and R'), these compounds can be divided in three different groups.

The first group encloses the azo-moieties with aromatic substituents, that have a stabilizing conjugation effect on the (very) reactive nitrogen double bond. These products became very popular during the Industrial Revolution, especially for their application as colourant and pigment. However, when treated under reductive circumstances, the unstable azo-bond can be broken with the release of aromatic amines. Since some of these amines possess a carcinogenic character, a European ban was established on the use of colourants based on di-azo molecules.[4,5]

Replacing the aromatic substituents by their aliphatic counterparts in azo compounds, lowers the stabilization degree of the N=N bond. This leads to molecules that disintegrate, at higher temperature, into radicals and nitrogen gas. The best known example in polymer

science is azobisisobutyronitrile (AIBN), a well-established radical initiator that is used in radical polymerizations.[6] If required, the released nitrogen gas can be used as foaming agent (for instance in the production of thermoplastics[7]). Using this type of components requires some caution, especially when the nitrogen content of the used moieties is high, due to the explosive nature.[8,9]

The last group of azo compounds consists of the azodicarboxylderivatives, with general formula R−CO−N=N−CO−R' where the carbonyl groups are in conjugation with the azobond, stabilizing the latter. However, the electron withdrawing effect is the main responsible for the lowering of the *lower occupied molecular orbital* (LUMO), giving these azodicarboxylates a very high reactivity.

Although the reactivity of azodicarboxylates is already high, it can be further increased by getting inspiration out of the classical reactivity pattern of fumaric acid – maleic acid – maleic anhydride (where the N=N is replaced by a C=C bond).[10] This shows that the carbon-carbon double bond gets a higher reactivity when the *trans*-configuration is replaced by a *cis*–configuration. Maleic anhydride has the highest reactivity due to the five-membered ring that contains a strained cis–conjugated double bond. When this pattern is transferred to the azodicarboxylates, the same trend is perceived (see Figure II.1).

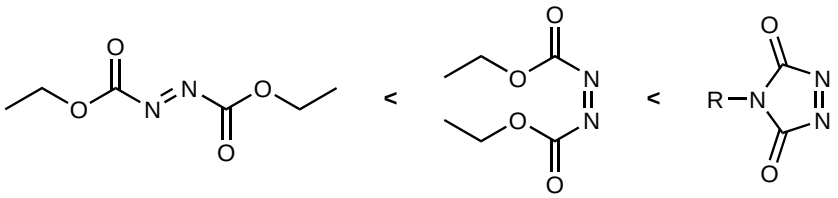
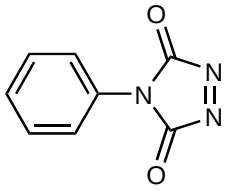


Figure II.1: Reactivity pattern for dicarboxylates. There is an increase in reactivity by replacing the *trans* conjugated double bond to a *cis* conjugated double bond, the highest reactivity is obtained by fixing the *cis* conjugation by means of a cyclic structure.

The cyclic azodicarboxylate 4-aryl-1,2,4-triazoline-3,5-dione (TAD) was first reported by Thiele and Stange in 1894.[11] TAD's are heterocyclic azodicarbonyl compounds with a strained azo-bond. This bond is the reason of the high reactivity of TAD making reactions involving these moieties mostly high yielding and fast.[12,13] Besides the high reactivity, the azobond is also responsible for the chromophore behaviour of these TAD's where the electronegativity of the substituents determines the colour of the end product.[14] The first TAD molecule that Thiele and Stange could isolate (although not absolutely pure) was

a dark red powder that later on could be described as 4-phenyl-1,2,4-triazoline-3,5-dione (PhTAD (**1**) – see Figure II.2).

(a)



1

(b)

Figure II.2: a) 4-phenyl-1,2,4-triazoline-3,5-dione (PhTAD, **1**) as dark red crystals and b) chemical structure of PhTAD.

Since the substituent of the TAD's can only be varied on the *4*-position (R-group - see Figure II.1), one can expect all the molecules to have a similar, well distinct (red) colour. In many cases this provides a visual feedback mechanism for reactions involving TAD, where the appearance or disappearance of the colour forms an indication for the formation or reacting of TAD.

When browsing the relevant literature concerning the known practical synthesis methods for these TAD compounds, it becomes apparent that these involve, in most cases, the oxidation of the corresponding 1,2,4-triazolidine-3,5-dione ('urazole'). Therefore a first section (II.2) will briefly describe the synthesis of urazoles and their subsequent oxidation to triazolinediones.

TADs are highly activated species and can actually participate in a large variety of reactions, depending on conditions and reaction partners. The most important reaction partners (and posssible side reactions), for this doctoral work, are discussed in section II.3, followed by two case studies of this reactivity in relevant application areas. The first case study is a comprehensive overview of the use of triazolinediones in the field of polymer science (section II.4), while the second will discuss the use of these compounds in *click-like* applications (section II.5). This last section is of great importance to build a framework for the work provided in this thesis.

II.2 Synthesis of triazolinediones

Since in this doctoral work, the focus lies on the reactivity of TADs and less on the synthetic pathways, only a brief overview of the most relevant synthetic routes will be provided here. For a more complete overview, the interested reader is referred to our review article.[15]

The oldest synthetic routes for 4-substituted urazoles, used before the second half of the twentieth century, were all based on hydrazodicarboxamide intermediates or derivatives thereof.[11,16–21] The more efficient modern methods however are distinguished by the use of 1-alkoxycarbonyl semicarbazides (**2**) as alternative (and more activated) urazole (**3**) precursors. These key intermediates for urazole synthesis are commonly referred to as 'semicarbazides'. As semicarbazides themselves can be assembled in a number of efficient ways, only the most relevant, for this doctoral thesis, will be discussed here. In a last step the obtained urazoles could then be oxidized to the corresponding TADs (**4**) as can be seen in Figure II.3.

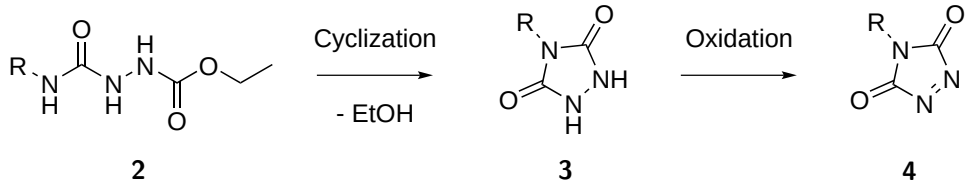


Figure II.3: The cyclization of a semicarbazide (**2**) leads to the corresponding urazole (**3**) that can be oxidized to yield the desired TAD molecule (**4**).

II.2.1 Urazoles via semicarbazides

In 1961, more than seven decades after Thieles original synthesis, Zinner and Deucker were the first to propose a synthetic scheme for urazoles that actually improved upon the methods based on Thiele's original approach.[22] Rather than using biurea starting materials, they synthesized 4-phenyl- and 4-butyl urazole through cyclization of the corresponding 4-substituted ethoxycarbonyl semicarbazide, which only requires mild reaction conditions. Significantly, semicarbazides can be generated by plainly mixing an isocyanate with ethyl carbazate (**5** - in itself a readily available condensation product of cheap hydrazine and diethylcarbonate). While this new two-step approach is characterized by significantly

higher overall yields and much milder reaction conditions, it took ten years for this synthesis to gain the attention of the wider scientific community. Cookson and co-workers were the first to publish an efficient synthetic procedure for 4-phenyl-1,2,4-triazoline-3,5-dione starting from hydrazine, diethyl carbonate and phenyl isocyanate in 1971.[23] This procedure includes the generation of ethyl carbazate (**5**), the subsequent reaction with phenyl isocyanate and cyclization to obtain 4-phenyl urazole – according to Zinner and Deucker – and the final oxidation to the triazolinedione. As this sequence was widely adopted thereafter, it became generally known as the *Cookson method*,[24] and triazolinediones became regularly referred to as *Cookson reagents*.[25–27] The Cookson method for urazole synthesis has found many applications, where the main differences are found in synthetic strategies to obtain the semicarbazides as can be seen from Figure II.4.

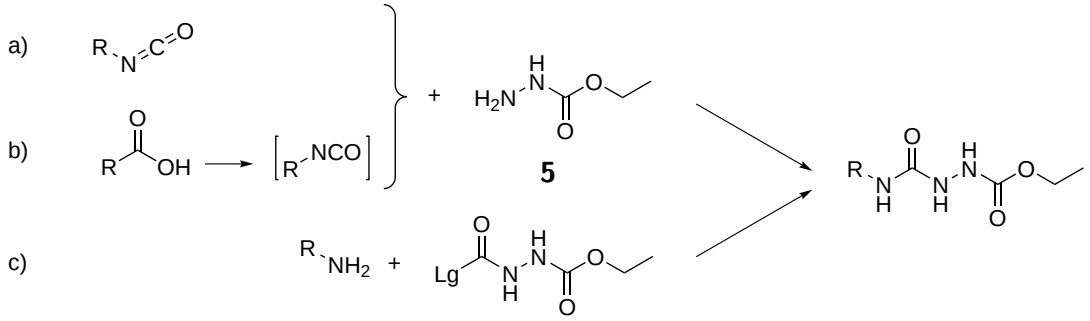


Figure II.4: Starting compounds to yield semicarbazides: a) isocyanates, b) carboxylic acid and c) amines.

II.2.1.1 Semicarbazides from isocyanates

As a result of the high reactivity of isocyanates, these readily react at room temperature with ethyl carbazate (**5**) to give the corresponding semicarbazide adducts in excellent yields, typically after stirring overnight. If the reaction mixture is heated, complete conversions can be achieved in a matter of hours. The role of the solvent is not critical, but as the semicarbazide products tend to precipitate from hydrophobic solvents such as toluene, the use of such solvents vastly simplifies the workup to the point that after collecting and drying the solids, there is generally no more need for a further purification step.

Although the original Cookson method was mainly used for the synthesis of 4-phenyl semicarbazides (and urazoles) from phenyl isocyanate,[22,23,28,29] the substrate scope was quickly expanded to include a wide range of isocyanates as well. So it became apparent

that the structural variations in the obtained semicarbazides was only limited by the availability and reactivity of the isocyanates themselves.[30] This means semicarbazides bearing a chemical functionality for further modifications are not easily accessible by this strategy because of the general incompatibility with isocyanates.

The Cookson method can also be used for the preparation of bis-semicarbazides when the corresponding diisocyanate is used. The possibility to convert typical polyurethane monomers, such as 4,4'-methylene diphenyl diisocyanate (MDI),[31] into the corresponding bivalent triazolinedione (bisTAD) reagents obviously attracted the interest of polymer chemists. Early reports by Wald and Wamhoff as well as Butler and co-workers triggered the development of a wide range of difunctional semicarbazides (and TAD reagents).[32–36] These bivalent compounds also attracted attention in the patent literature, with reports on the transformation of some of the industrially relevant diisocyanates.[21]

II.2.1.2 Semicarbazides from carboxylic acids

Despite the ease and versatility of the Cookson method, typically accompanied by a high yield of the overall synthesis, the method is limited by the structural variety in commercially available isocyanates. Therefore, a lot of research has been performed to obtain semicarbazides from alternative starting materials. As in fact isocyanate-free synthetic schemes have been developed (*vide infra*), most 'alternative' procedures are actually based on the Cookson method, where an isocyanate is prepared *in situ*, avoiding the isolation or purification of hazardous intermediates. Thus, the Cookson method has been extended to the preparation of semicarbazides from simple carboxylic acids.

A carboxylic acid can easily be converted into an acyl azide, which upon heating will readily rearrange into an isocyanate with the expulsion of nitrogen gas. The well-known *Curtius rearrangement* is a classical 19th century organic reaction,[37–39] but its first application for the synthesis of urazoles was only reported in 1990.[40] The acyl azide intermediates can be prepared in one step from carboxylic acids using an azidating agent such as diphenylphosphoryl azide (DPPA, **6**), or via an intermediate acid chloride that can be reacted with sodium azide (Figure II.5, bottom). While acyl azide intermediates can be isolated, the crude product or reaction mixture can be directly used for the Curtius rearrangement to the corresponding isocyanate. Upon completion of the Curtius

rearrangement, ethyl carbazate can simply be added to the reaction mixture, upon which the desired semicarbazide will precipitate.

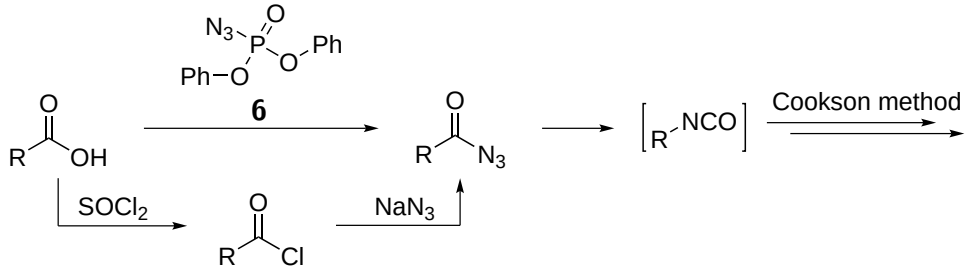


Figure II.5: Extension to the Cookson method by *in situ* generation of an isocyanate via the *Curtius rearrangement* of an acyl azide. The latter compound can be obtained in one step using diphenylphosphoryl azide or in two steps via an activated carbonyl derivative such as an acid chloride[25,41–44]. [45,46]

II.2.1.3 Semicarbazides from amines

In analogy with the synthesis of semicarbazides from carboxylic acids using a Curtius rearrangement, isocyanates can be readily obtained *in situ* by combining an amine - or its hydrochloride salt - with phosgene. After removal of gaseous hydrochloric acid, addition of ethyl carbazate to the resulting reaction mixture thus leads to the formation of semicarbazides. Later, the use of safer alternatives to the gaseous phosgene were demonstrated as well, i.e. trichloromethyl chloroformate (diphosgene)[47] and bis(trichloromethyl) carbonate (triphosgene).[48,49] The reaction of an amine with (di- or tri-)phosgene results only in the formation of the desired isocyanate and hydrochloric acid. In the absence of acid-sensitive moieties, this gas is easily removed and the isocyanate is obtained in essentially pure form, resulting in high yields of the target semicarbazide.[50] However, as a result of safety issues with regard to the use of isocyanates and especially phosgene, alternative isocyanate-free methods have been developed for the production of semicarbazides from amines, which altogether avoid the use and even intermediate formation of hazardous and less readily available isocyanates (as compared to amines and anilines).[51]

II.2.1.4 Cyclization

Most semicarbazides discussed above can be readily cyclized to the corresponding urazole. Thus, only a limited amount of protocols can be found throughout all reported syntheses. A clear rationale to why a certain method should be preferred over another one is rarely

provided. In general, however, the cyclization of a semicarbazide is achieved using mildly basic conditions in a protic solvent.[22,28,50,52,53]

The original Cookson method reports a simple treatment of the semicarbazide with an aqueous potassium hydroxide solution at reflux temperature to effect cyclization of the semicarbazide to the urazole. When the cyclization is completed, as a result of the acidity of the formed urazole,[54,55] the urazole is obtained as a water-soluble potassium salt (solvated urazolyl anion), which, upon acidification of the solution to pH $\approx$ 1-2, precipitates from the aqueous medium as a neutral compound.

A somewhat milder alternative to an aqueous potassium hydroxide solution is represented by sodium ethoxide in refluxing ethanol. This widely adopted method also allows for the isolation of urazoles in high yields, but longer reaction times of up to 24 hours have to be taken into account. Often, these reaction conditions are the best choice if the substituent on the urazole, such as an aliphatic residue, prevents it from precipitating from an acidified aqueous solution.[56,57] This is mainly because this procedure is compatible with a non-aqueous work-up to isolate the urazoles as an oil.

Potassium carbonate can also be used as a base, rather than the much stronger ones applied in the previous systems. As a result of these milder conditions, higher yields can be obtained with some problematic substrates although longer reaction times are necessary. This protocol can be used in both water as well as in alcoholic solvents.[52,56,58]

II.2.2 Oxidation of urazoles to their corresponding 1,2,4-triazoline-3,5-diones

An important remark in the context of TAD synthesis is that, although oxidation of urazoles is a very straightforward reaction, it can sometimes be a true bottleneck of triazolinedione synthesis. Although urazoles are in fact readily oxidized by most oxidants, these reactions are hard to perform because of two interrelated issues, i.e. the chemoselectivity of the oxidant and the reactivity of the resulting TAD compounds. Especially isolation of TAD reagents from reaction mixtures can be challenging. Ideal oxidation methods should thus be highly chemoselective, give one single triazolinedione reaction product, and generate no waste products or only waste products that are read-

ily removed. Both the oxidant and its reduced forms should not react with the TAD compound. Unfortunately, there are no generally successful approaches, and developing a successful protocol is often a question of trial and error, balancing the kinetics of several processes. For some applications, an *in situ* oxidation method is preferred, which avoids the isolation problem, but usually exacerbates the chemoselectivity problem. Due to the limited relevance of the used oxidation methods in this project only three important oxidations methods are described here. However a more complete overview of the oxidation to triazolinediones can be found in our review on triazolinediones.[15]

Stickler and Pirkle[28] developed a straightforward water- and acid-free protocol for the oxidation of urazoles by making use of gaseous dinitrogen tetroxide (N_2O_4), which is known to be in equilibrium with nitrogen dioxide ($NO_{2(g)}$),[59] which is a milder (reduced) and dehydrated form of nitric acid.[60] The use of dinitrogen tetroxide, which is a liquid below 20 °C, can convert urazoles to their corresponding triazolinediones in an almost traceless manner, as the gaseous oxidant can be used in excess without compromising the straightforward isolation procedure. The residues obtained from simple evaporation of the reaction mixture thus contain little contaminants and pure, bench stable TAD reagents can be obtained by a twofold sublimation of the residue. The toxic gas N_2O_4 can be handled in liquid form, but a solution of this gas in an inert solvent can also be employed to achieve less hazardous handling procedures.[47,61,62] This reaction can also be performed in more polar solvents such as ethyl acetate, which broadens the scope of the classical nitric acid procedure considerably, which is typically limited to (hydrophobic) chloroform- or dichloromethane soluble TAD reagents.[63] Today, the N_2O_4-based oxidation is still one of the most effective and reliable methods to obtain TAD reagents of a high analytical quality, owing to the straightforward work-up (removing of volatiles).

Besides gaseous dinitrogen tetroxide, a second major chemical class of oxidants used to convert urazoles into triazolinediones comprises halogens and their derivatives. Elemental chlorine and bromine often give low yields for urazole oxidations, presumably due to their limited chemoselectivity and because of the acid sensitivity of the formed TAD compounds. Cookson achieved a more controlled oxidation process by using *tert*-butyl hypochlorite for the oxidation of 4-phenyl urazole in dry acetone at low temperatures (−50 to −78 °C).[26] The low temperature most likely prevents side reactions with acetone,

which is not a completely inert solvent for triazolinediones.[64,65] Although isolation gave the resulting TADs in 80% yield, which can be directly used in a subsequent reaction, the obtained product was unstable, even after sublimation.[23] A few years later, quantitative yields were made feasible at room temperature by changing the solvent to dioxane, which allowed for a relative stable crystalline product to be isolated.[10]

In order to avoid the use and handling of hazardous (and harsh) elemental bromine and chlorine, a number of alternative reagents and procedures have been developed that rely on the slow generation of halogen-based oxidants. Moreover, a heterogenous character of an oxidation procedure can be achieved by simply using a solid supported reagent. This usually gives shorter reaction times and a more efficient removal of salts by filtration.[66] Recently, Zolfigol, Mallakpour and co-workers, searched for heterogeneous halogen-generating reagents that can be easily used in excess, and allow a straightforward work-up procedure by simple filtration and subsequent evaporation of the resulting reaction mixture. The authors reported on the use of trichloromelamine,[67] N,N-2,3,4,5,6-heptachloroaniline[68] and 1,3,4-6-tetrachloro-3a,6a-diphenylglycoluril (iodogen)[69] as a source for active chlorine species and N,N,N',N' tetrabromobenzene-1,3-disulfonamide,[67] hexamethylenetetramine bromine,[70] 1,2-(dipyridiniumditribromide)-ethane[70] and tribromoisocyanuric acid[70] to generate active bromine species *in situ*.[70] The tetrameric complex of 1,4 diazabicyclo[2.2.2]octane and bromine (DABCO-Br, see Figure II.6 - **7**)[70] has been found to be a particularly useful heterogeneous reagent, which does not require the addition of silica gel to remove excess reagents and byproducts. Due the mild reaction conditions, this bromine complex was used as the oxidizing agent of choice in the following doctoral work.

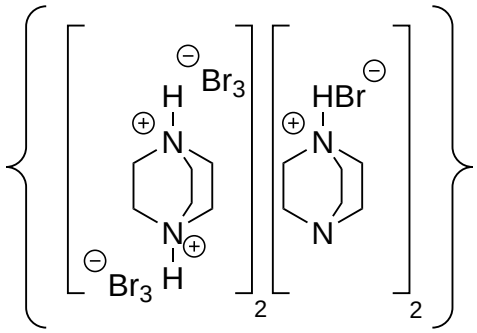


Figure II.6: The tetrameric complex of 1,4 diazabicyclo[2.2.2]octane and bromine (DABCO-Br) (**7**), the oxidizing agent that will be used in this thesis.

II.3 Overview of the reactivity of TAD molecules

TAD reagents have an overall resemblance in chemical structure with the more widely known maleimides, which are a well-established class of important synthetic tools for a wide range of applications, including *click* chemistry.[71–73] Indeed, also their modes of reactivity show some important similarities. Nevertheless, TAD compounds react much faster than maleimides and can also participate in a larger variety of pericyclic reactions with a much wider range of substrates - and with simple olefins in particular.[14,74,75] TAD reagents also have a higher intrinsic thermodynamic driving force than maleimides. Thus, whereas many maleimide-based conjugation reactions are reversible processes, most TAD-based reactions are completely irreversible. An important difference is the relative lack of (controlled) reactivity TAD reagents show towards typical nucleophiles such as amines and thiols.

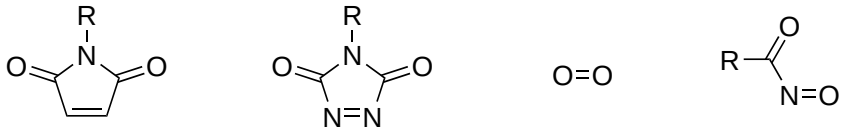


Figure II.7: TAD reagents and related reactive compounds: maleimides, triazolinediones, singlet oxygen and nitrosocarbonyls.

In terms of reactivity, TAD compounds have often been compared with singlet oxygen.[76–78] Indeed, both singlet oxygen and TADs show a great preference for Diels-Alder, *ene*-type, and (2+2)-cycloaddition reactions for more or less the same range of substrates (electron rich or non-polarized olefins). This similarity in reactivity can also be related to a correspondence in the particular arrangement and energies of the frontier orbitals (HOMO and LUMO), with a filled and an empty π-type orbital of very similar energy (*vide infra* chapter III).[79] In essence, TAD and singlet oxygen have a strong preference for orbital-controlled reactions and favour delocalized π-cloud type substrates over typical localized or ionic nucleophiles. A major difference in terms of practicality between singlet oxygen and TAD reagents, however, is their lifetimes. Many TAD compounds can be isolated and stored for longer times (*vide supra*), while singlet oxygen only has a half-life of a few microseconds in most organic solvents. Moreover, TAD reagents offer the possibility to introduce a wide range of functionality (R-groups), instead of just effecting oxygenations. A reagent that is – in more than one way – a kind of hybrid of TADs

and singlet oxygen, are the nitrosocarbonyl compounds.[79,80] These also have very limited life times, and need to be generated *in situ* just like singlet oxygen, but can be used to introduce different substituents. Recently, nitroso compounds are receiving a renewed interest as versatile synthetic tools for various applications.[81]

The preferred reaction partners and reactivity modes of TAD reagents are outlined in Figure II.8. Each of these reaction types will be briefly discussed below, in dedicated subsections.[12] Apart from these very fast conjugation reactions in which carbon-nitrogen bonds are formed, TAD reagents can engage in a wide range of other reactions. These secondary reaction modes are often much less selective but also much slower than the ones shown in Figure II.8. Nevertheless, these reactions can be observed as undesired side reactions, and will also be briefly discussed in this section (II.3.5). A more detailed overview of the reactivity of TAD molecules, can be found in review articles.[12,64,82–84]

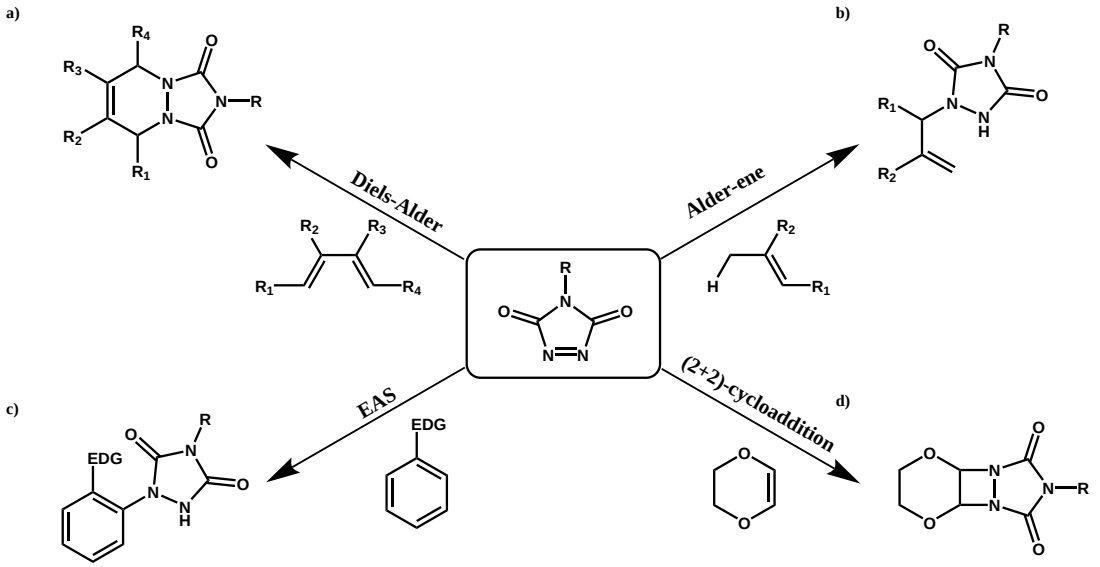


Figure II.8: Reactivity of TAD molecules: a) Diels-Alder reaction, b) Alder-*ene* reaction, c) Electrophilic Aromatic Substitution (EAS) with an activated aromatic system (EDG = Electron Donating Group) and d) (2+2)-cycloaddition.

II.3.1 Diels-Alder reactions

The original discovery of the Diels-Alder (DA) reaction is actually closely linked to the chemistry of azodicarbonyl compounds. Since its discovery, the DA reaction has been developed into one of the most efficient and widely applicable organic bond-forming reactions. The DA reaction allows the introduction of two new carbon-carbon σ-bonds and

up to four new stereocentres,[85] with very pronounced and predictable levels of chemo-, stereo- and regioselectivity. Generally, the reaction requires elevated temperatures, but many DA reactions can also be effected at low temperature by using simple catalysts.[86] The DA reaction is a highly atom-economical process, and at least in theory, the bond forming process can be reversed, giving a retro-Diels-Alder (rDA) reaction that releases the original reaction partners.[87] This dynamic feature of the DA/rDA reaction has been used in a range of interesting applications in organic synthesis such as temporary protection of dienes,[88,89] scavenging of dienes from complex reaction mixtures,[90] and capturing and releasing transient reaction intermediates.[91] In polymer chemistry, the rDA reaction has been used to design covalently adaptable materials that show interesting properties such as healing[92] and remendability.[93]

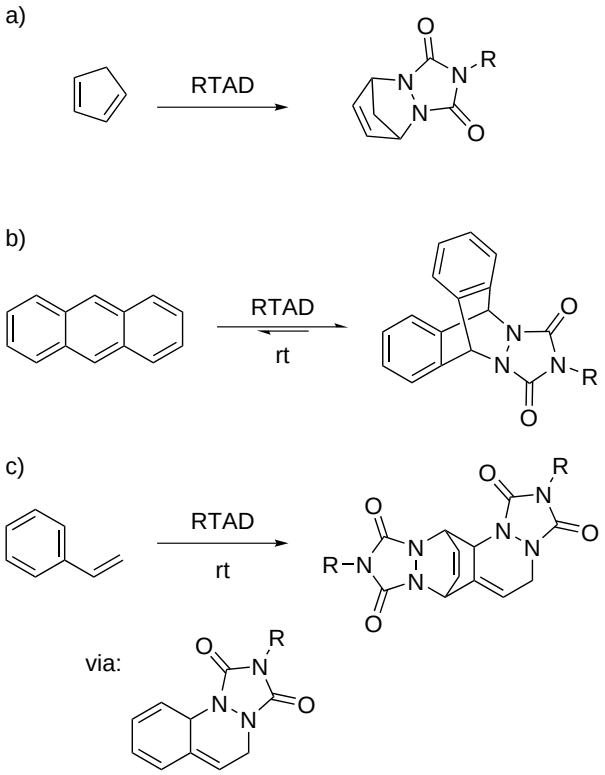


Figure II.9: a) 1:1 adduct of TAD and cyclopentadiene, b) 1:1 adduct of TAD and anthracene (reversible at room temperature) and c) 1:2 adduct of TAD and styrene (TAD and styrene react to form a diene that reacts *in situ* with another TAD moiety).

The DA reaction has an enormous substrate scope, but TAD compounds were relatively late entries to the Diels-Alder toolbox. However, since Cookson's original report of an instantaneous reaction between PhTAD and cyclopentadiene at −78 °C,[26] TADs have become an intensively studied class of DA substrates, acquiring the reputation of being the

fastest dienophile that can be isolated.[12,79] The exceptional reactivity of triazolinediones can be appreciated by the fact that their reaction with 'good' Diels-Alder dienes is almost instantaneous and quantitative even at quite low temperatures (−78 °C to −50 °C) (Figure II.9a). Reactions with (much) less reactive dienes, such as anthracene or styrene, still proceed smoothly at room temperature (Figure II.9b and c). The reaction between anthracene and TAD is very fast at room temperature, but it is also one of the few TAD DA reactions that shows reversibility at room temperature,[74] related to the aromatic stability of the diene. In the case of styrene, which does normally not react with dienophiles even at elevated temperatures, a 1:2 adduct is quantitatively formed via a highly reactive diene intermediate that cannot be isolated (Figure II.9c).[10] Reaction of styrene with just one equivalent of a TAD compound thus leads to a clean conversion of half of the styrene into the bis-adduct with TAD.

II.3.2 Alder-*Ene* reactions

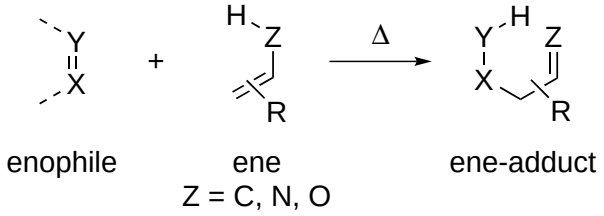


Figure II.10: General reaction scheme of an Alder-ene reaction.

The Alder-*ene* (AE) reaction (also referred to as *ene* reaction) can be defined as the reaction of an alkene bearing an allylic hydrogen (the *ene*) with a double bond (enophile) and was first described by Kurt Alder in 1943 (Figure II.10).[94] During his Nobel lecture in 1950 it was classified as an 'indirect substitution addition' or '*ene* synthesis'.[95] It belongs to a general class of pericyclic reactions and comprises the migration of a σ-bonded hydrogen atom, the formation of a new C-C σ-bond on expense of a C-C π-bond and the displacement of the initial π-bond.[96]

Despite the great potential in organic synthesis of the *ene* reaction,[97] the applications of the AE reaction have been rather limited as compared to the DA reaction. One reason for this is the unfavourable activation entropy and enthalpy, related to the highly ordered transition state with relatively poor orbital overlap, which results in much slower reaction rates.[98] Indeed, the AE reaction often requires extreme conditions (high pressure

and temperature close to or above 200 °C), especially in the case of intermolecular *ene*-reactions. The use of Lewis acid catalysts can lead to much enhanced reaction rates at lower temperature, but the issue of regioselectivity, in the common case where more than one allylic hydrogen is present in the ene substrate, has also limited such methods mostly to intramolecular applications (*ene* cyclizations).[99] The introduction of highly reactive enophiles such as TAD compounds, however, have opened the door to quite reliable and even selective intermolecular *ene* reactions (Figure II.11). Just a few years following Cookson's isolation of TAD compounds, its use as a potent *enophile* was found in room temperature reactions with simple alkene substrates, giving N-allylurazole adducts in quantitative yields.[100] A number of more recent illustratives TAD-based AE reactions are shown in Figure II.12.

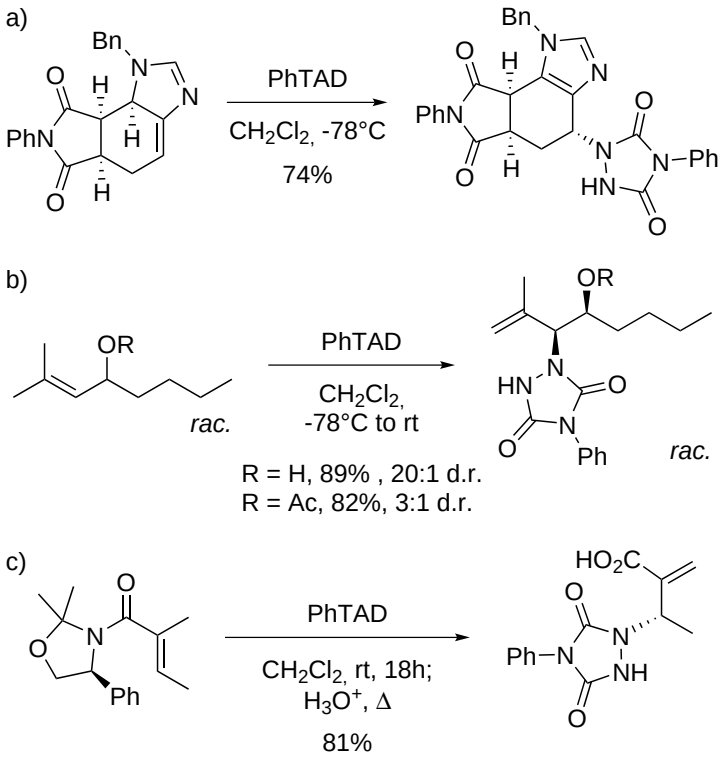


Figure II.11: a) DA-cycloadducts of 4-vinylimidazoles are viable substrates for high yielding and highly diastereoselective *ene* reactions with TAD[101], b) regio- and diastereoselective *ene* reaction of 4-phenyl-1,2,4-triazoline-3,5-dione (PhTAD) with chiral allylic alcohols[102] and c) reaction between 2,2-dimethyloxazolidines and PhTAD.[103]

The mechanism of the TAD-based *ene* reactions has been a matter of some debate in the literature, where a six-electron concerted pericyclic process has been discarded in favour of a stepwise route involving the formation of a *zwitterion aziridinium imide* (AI, Figure II.12).[104,105] The exact mechanism for subsequent hydrogen transfer is unclear but

likely involves an open form *polarized diradical* (PD) intermediate, and might actually be solvent-dependent.[106,107]

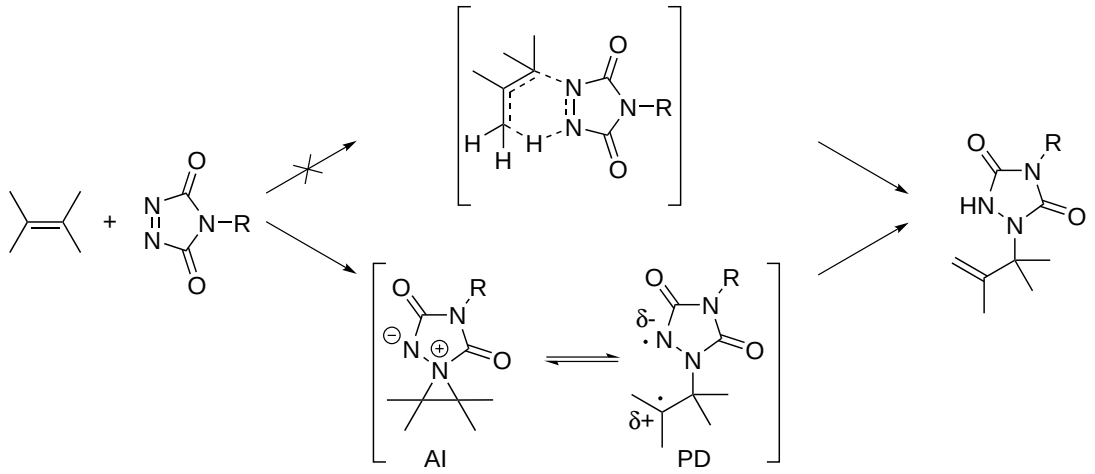


Figure II.12: Alder-*ene* reaction between a mono-olefin and a TAD molecule. Two mechanisms are depicted: a) a concerted pericyclic process via a six-membered ring transition state and b) a stepwise route (via an open zwitterion (OZ) or an aziridinium (AI) transition state).

Because of the (very) high activation barriers for typical *ene* reactions, it is not unexpected that the retro-Alder *ene* (rAE) reaction has only rarely been observed in comparison to the retro-DA reaction.[97,108] Most of the described thermoreversible AE reactions require pyrolysis-type conditions and are thus of limited value in synthetic applications.[97,109] Based on the much higher kinetic reactivity of TAD compounds as enophiles, one might expect to find *ene* reactions with these reagents that can be reversible in reasonable temperature intervals. However, so far only one example has been reported in literature. Baran and Corey described in 2003 the possibility to thermoreversibly protect an indole functionality.[76] Indoles readily and selectively form ene adducts even at 0 °C. By simply heating the adduct, a rAE reaction will take place, which allows removal of a volatile TAD reagent, and gives the indole moiety (Figure II.13).

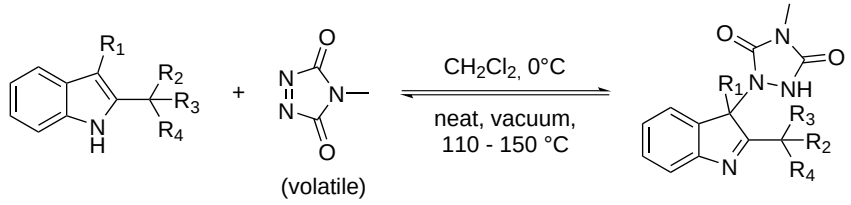


Figure II.13: The 2,3-π bond of certain indoles can be protected via the *ene* reaction with a TAD compound. Heating the adduct under vacuum allows for deprotection by removal of the volatile MeTAD.

II.3.3 Electrophilic aromatic substitutions

In *Electrophilic aromatic substitution* (EAS) reactions, TAD compounds can act as suitable electrophiles for highly activated aryl systems. Because TAD compounds are neutral, the expected carbocationic intermediates are actually zwitterions that can also exist as an *aziridinium imide* (AI) intermediate (Figure II.14a). This initial addition intermediate needs to undergo a proton transfer that yields a 1-aryl substituted urazole compound. Although this mode of reactivity of TAD compounds can be quite pronounced, surprisingly, only a handful of studies have been reported on this reaction.[12,110–115] Suitable substrates include dialkoxy- and trialkoxy-substituted aryls, as well as electron rich nitrogen-containing aryls such as various aniline derivatives and indoles. The formation of charge-transfer complexes has also been observed in these reactions, although it is unclear if they are involved in the reaction pathway.[116] Quite recently Breton *et al.* were able to expand the substrate range for less activated aryl substrates (including some dialkyl-substituted benzenes) by using trifluoroacetic acid[117] as a catalyst or by simply shining visible light[116] on the reactions.

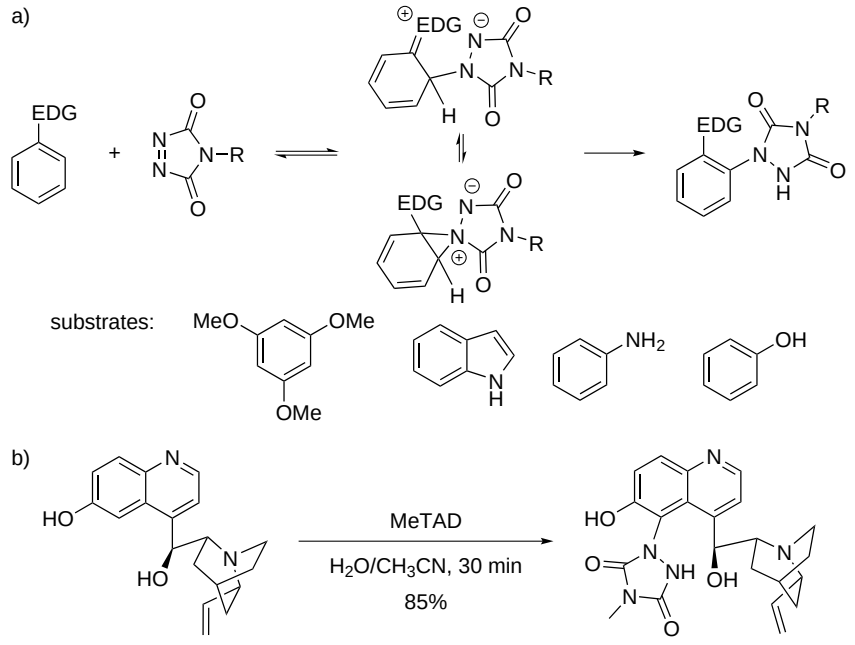


Figure II.14: a) Electrophilic aromatic substitution between an electron enriched aromatic system and a TAD molecule. b) Example of a selective formation of an elaborate 1-aryl-4-methyl urazole in aqueous conditions.[118]

The reaction of 2,3-disubstituted indoles has been discussed under *ene* reactions, but it can also be considered as an 'aborted' or shunted EAS reaction of TAD with an in-

dole substrate, wherein the rearomatisation step is prevented. Likewise, the reaction of phenols with TAD compounds can be considered as an *ene* reaction,[119] followed by a keto-enol tautomerization to restore aromaticity. The phenolic proton indeed seems to be implicated in the EAS reaction with TAD, as simple alkoxy-aryls are much less reactive substrates,[116,117] while phenols are excellent substrates, especially in aqueous medium, that give selective and rapid formation of aryl-urazoles (Figure II.14b).[110,116,118]

II.3.4 (2+2)-Cycloadditions

In classical pericyclic reactions, (2+2)-cycloadditions usually constitute thermally forbidden processes that require photochemical conditions, because of the requirement for both π-bonds to approach each other in an antarafacial way, which is sterically impossible. However, for sterically more accessible two-electron π-systems, such as ketenes,[122,123] an antarafacial addition mode is possible and orbital symmetry-allowed thermal concerted (2+2)-cycloaddition can occur. Indeed, also with TAD reagents a thermal, antarafacial (2+2)-cycloaddition is geometrically possible. Although a concerted thermal (2+2)-cycloaddition cannot be excluded for reasons of orbital symmetry, Seymour *et al.* showed that the cycloaddition reaction actually proceeds via a stepwise *aziridinium imide* (AI) intermediate that rearranges to the neutral diazetidine ring (Figure II.15a).[120] Especially electron rich alkenes are good substrates for this TAD (2+2)-cycloaddition, but these

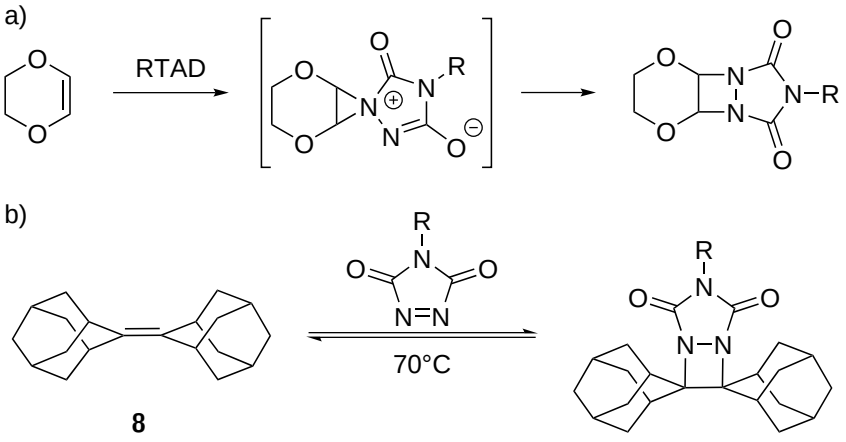


Figure II.15: Examples of (2+2)-cycloadditions with TAD compounds. a) The reaction with 1,4-dioxene proceeds according to Seymour *et al.* via an aziridinium intermediate state.[120] b) Synthesis of a diazetine via the reaction of MeTAD with phenyl vinyl sulfide, followed by oxidation and pyrolysis of the labile adduct.[121] c) The reaction of TAD with adamantylideneadamantane (**8**) is reversible at elevated temperature.[120]

should not possess allylic hydrogens that can be transferred to give the thermodynamically preferred *ene*-type adducts. Indeed, the resulting diazetidine rings are highly strained compounds. Seymour studied the reaction of adamantylideneadamantane (**8**), which does not have any transferable allylic hydrogens, and found that the (2+2)-cycloaddition is actually reversible at slightly elevated temperatures (Figure II.15b).[120]

II.3.5 Secondary reaction modes of TADs and important side reactions

As we have seen in the previous sections, TAD compounds are highly reactive towards electron rich delocalized π-cloud type substrates, including simple alkenes. In the case that there are no such suitable reaction partners available, TAD compounds can undergo a whole range of other reactions, albeit with slower kinetics. Thus, often these reactions are observed as undesired side reactions or as decomposition reactions. The main types of these 'slow' TAD reactions are summarized in Figure II.16.

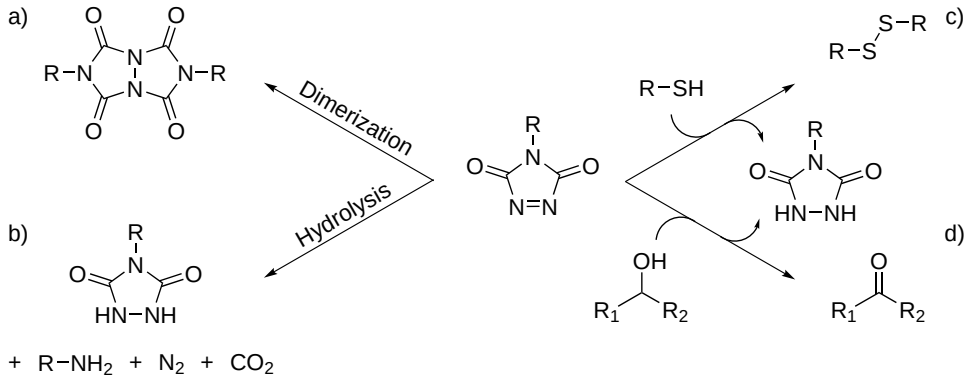


Figure II.16: Important side reactions involving TAD. a) Dimerization of TAD under UV irradiation or when heated above 150 °C, b) hydrolysis of TAD, c) oxidation of thiols and d) oxidation of alcohol to aldehydes or ketones.

As already mentioned, some TAD molecules are not stable enough (or too reactive) to be isolated. However, in crystalline form, reagents such as PhTAD are relatively stable up to 160 °C and can be stored for several months in a dark, cold environment without significant decomposition.[10] However, when PhTAD is exposed to UV irradiation (for a longer period of time) or to temperatures above 160 °C, dimerization through self-condensation occurs (Figure II.16a).[124] Thus, this dimerization process – actually the loss of nitrogen gas – puts an upper limit on reaction temperatures that can be employed when working with TAD reagents.

Although quite a few reactions of TAD compounds are actually conducted in water as solvent,[110,111] hydrolysis of TAD is a feasible process.[74] When stored in water for longer times, TAD compounds will slowly hydrolyze, giving the corresponding urazole and amine as final decomposition products (Figure II.16b). This problem can usually be avoided by storing TAD compounds in a dry medium and container. The presence of acid or base can accelerate the hydrolysis, which needs to be taken into account when working in non-neutral media.

As can be expected from section II.2, TAD compounds are also redox active compounds, acting as oxidants for substrates such as thiols[125], leading to disulphide formation (Figure II.16c). Also alcohols can be oxidized (Figure II.16d).[126] In some cases, these mild oxidation reactions are actually synthetically useful, giving a clean oxidation under relatively neutral conditions.

In spite of all the different modes of reactivity that are available to TAD compounds, most TAD reactions of the type described in the previous sections are surprisingly chemo-, regio- and even stereoselective, also in the presence of competing reaction partners. This is because of the very pronounced kinetic selectivity TAD reagents have. Another feature that contributes to this orthogonal behavior of TAD reagents, is the fact that although it is a highly electrophilic species, it reacts only very slowly with classical (ionic) nucleophiles with 'localized' electron density. A notable chemoselectivity issue in TAD reactions can arise in the presence of basic amines. The reaction of TADs with basic amines depends on a number of factors, and also a distinction has to be made between primary, secondary and tertiary amines. In combination with primary and secondary amines, TAD compounds can promote an acylation-type reaction of the amine, ultimately giving a urea derivative (Figure II.17a).[127] The reaction proceeds via a nucleophilic attack of the amine that leads to the ring-opened species, which quickly expels N_2 and CO gas with the formation of a urea. In the case of tertiary amines, the reaction follows a different course after initial ring opening, and can react with another TAD molecule. The original amine is then expelled to form the dimer as can be seen in Figure II.17b, and thus acts as a TAD-dimerization catalyst.[128] These side reactions can be suppressed by prior protonation of the amines (e.g. by adjusting the pH of the reaction medium), or by using substrates that are (much) more reactive than amines (such as Diels-Alder-type dienes). Anilines

are usually less problematic, and mostly give EAS reaction with TAD, without significant urea formation or TAD dimerization.

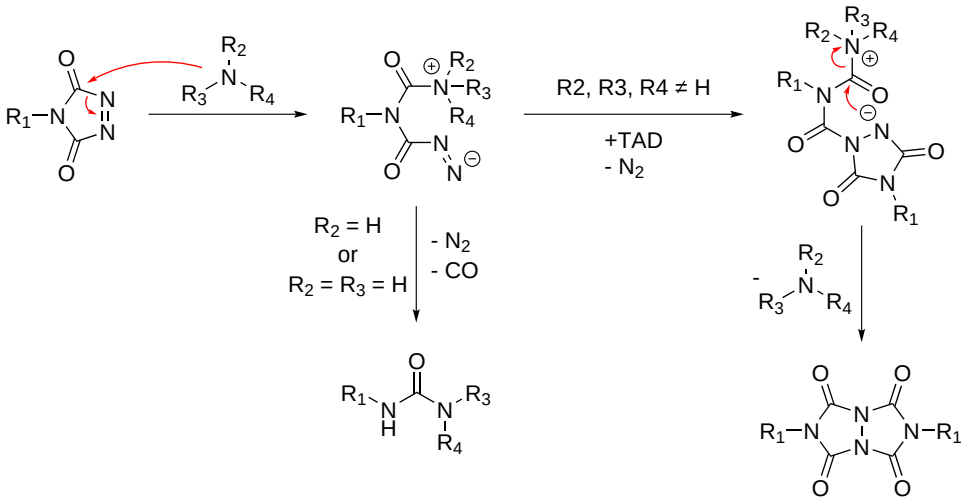


Figure II.17: Reaction mechanism of the unwanted side reaction between TAD and primary/secondary amines or tertiary amines.

II.4 The use of 1,2,4-triazoline-3,5-diones in polymer science

Following the introduction of TAD-compounds as versatile reagents in organic synthesis in the 1960s, the polymer community also developed an interest for the unique reactivity of TADs. Most of the early literature appeared in the 1970s and mainly dealt with the modification of polydienes. This particular subject has been covered in two dedicated reviews by Butler in the early 1980s.[64,82] Nevertheless, the use of TADs in polymer chemistry has been further explored in various other macromolecular applications and thus a comprehensive overview involving this work is given below.

II.4.1 Homopolymerization of TAD-based monomers

In 1970, Pirkle and Stickler investigated a direct polymerization reaction of TAD-based molecules, to obtain polymers with an exotic all-nitrogen backbone (Figure II.18).[129] In this report, a 0.3 M carbon tetrachloride solution of 4-butyl-1,2,4-triazoline-3,5-dione (BuTAD) was irradiated with a halogen lamp for 8 minutes, giving a colourless polymer with an average molecular weight of 4200 g/mol (around 20 monomer units). However,

the obtained polymer had a very limited life time in the original CCl_4 solution, i.e. depolymerization slowly occurs within a timeframe of 30 minutes to a few days. Moreover, the polymer is fully degraded within minutes in the presence of trace amounts of pyridine. The same experiments did not give polymers when aromatic TAD components were used.

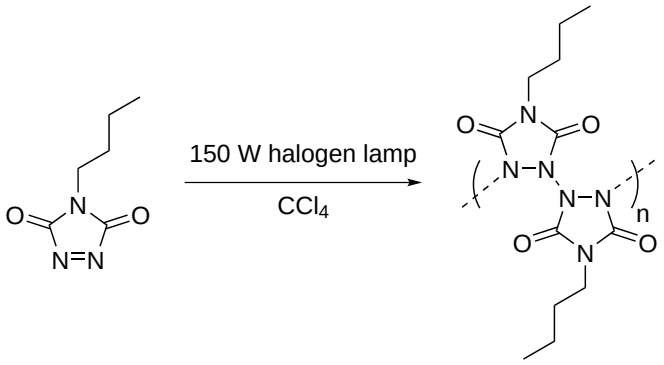


Figure II.18: Homopolymerization of BuTAD (n ≈ 10).

In an alternative approach to directly polymerize TAD compounds, Turner *et al.*[130] attempted the polymerization of bifunctional TAD molecules with the aid of sodium cyanide as catalyst, but no polymers were formed. It was not until Butler *et al.* studied the peculiar decomposition behaviour of (bifunctional) TAD molecules that homopolymerization of bisTADs was detected in 1985.[36] Indeed, in the presence of catalytic amounts of pyridine, a solution of a bifunctional TAD molecule in 1,2-dichloroethane can be gradually converted to a polymeric structure over the course of in 30 minutes to one hour. The polymerisation reaction, as judged by the disappearance of the characteristic TAD colour, was also found to be slower when it was conducted in the dark. The obtained polymers were fully characterized by IR and NMR to support their assigned structure shown in Figure II.19 and were also shown to have a remarkable thermal stability (decomposition around 300 °C). Up to now, this remains the only successful homopolymerization of TAD-containing molecules.

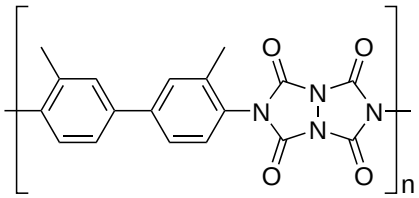


Figure II.19: Assigned structure of the obtained product of the polymerization of 3,3'-dimethyl-4,4'-bis-(1,2,4-triazoline-3,5-dione) diphenyl with the aid of catalytic amounts of pyridine.

II.4.2 Copolymerization of monofunctional TAD-monomers

TAD molecules possess a very particular reactivity towards a wide range of relatively simple reaction partners, supplying a range of different mechanisms as possible tools for a copolymerization. An interesting feature of TAD is its powerful electron-acceptor activity. Based on this, shortly following the work of Pirkle and Stickler,[129] Butler *et al.* showed that 4-phenyl-1,2,4-triazoline-3,5-dione (PhTAD) could react with a variety of electron-donating alkenes to yield alternating copolymers. A first example was the combination with vinyl ethers.[131] The reaction of PhTAD with ethyl vinyl ether in dichloromethane (DCM) resulted in a copolymer with a rather low M_n (up to 2400 g mol^{-1}), via a coupling of 1,4-dipolar intermediates (Figure II.20a) most likely involving a cationic ring opening-type polymerisation of the initially formed zwitterionic aziridinium-type adducts. When using a divinyl ether, a mixture of the expected copolymer and a (2+2)-cycloaddition adduct was observed. The yield for the copolymer could be increased by raising the temperature or switching to DMF as a solvent.

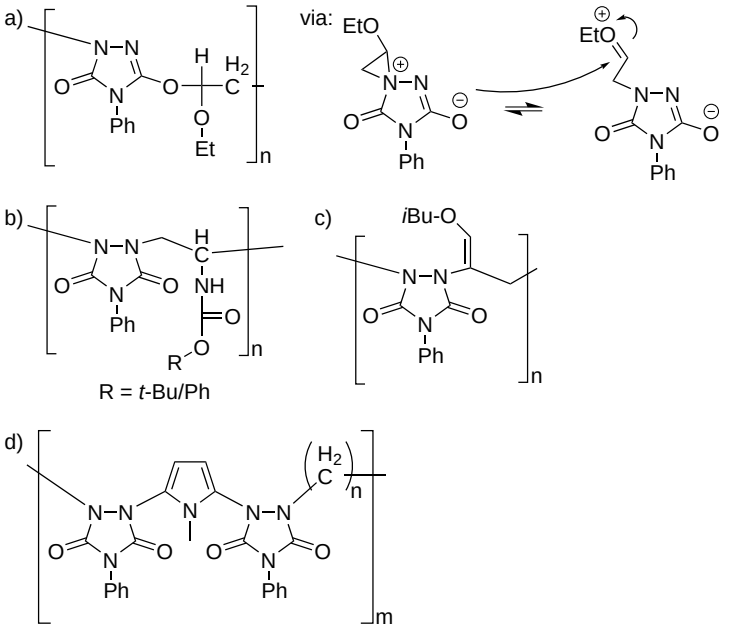


Figure II.20: Assigned structure of the obtained products via the polymerization of 4-phenyl-1,2,4-triazoline-3,5-dione with a) vinyl ethers, b) N-vinyl carbamates, c) isobutoxyallene or d) alkyldihalides.

In a follow-up study, Butler *et al.* investigated the copolymerization of PhTAD with N-vinyl carbamates.[132] More specifically, tert-butyl N-vinyl carbamate reacted spontaneously with PhTAD in DCM to form an low molar mass copolymer (M_n = 1000 g/mol).

However, in contrast to the results with vinyl ethers, this time proof was obtained that the reaction proceeded through the -N=N- bond rather than through the -N=N-C=O- system (Figure II.20b), as was previously stated. Similar results were obtained for reaction with N-vinyl carbazole.[132]

A final example of a polyaddition using monofunctional TAD-monomers was presented by Endo *et al.*, who copolymerized PhTAD with isobutoxyallene.[133] This highly exothermic reaction was performed at lower temperature (-40 to $-20\,^{\circ}\mathrm{C}$), resulting in polymers with M_n up to $10\,000\,\mathrm{g\,mol^{-1}}$ ($Đ \leq 1.72$ - Figure II.20c). These authors also studied the reaction mechanism, which can be either a classical free radical polyaddition process, or a stepwise ring opening-type mechanism involving zwitterionic intermediates. It was shown that the active species of the copolymerization was in fact a zwitterion and not a diradical. In case of using polar and donative solvents, the zwitterion is effectively stabilized, resulting in a higher yield and M_n as compared to polymers prepared in DCM. However, when the polymerization is performed in bulk, the role of diradical active species could not be excluded in this work.

In a variation on the above described strategy, Mallakpour *et al.* first reacted two PhTAD molecules with N-methylpyrrole.[134] The expected 2:1 adduct, obtained via a double EAS, was then deprotonated to its potassium dianion salt. A polycondensation of this dianion salt with alkyldihalides (1,2-dibromoethane, 1,2-diiodoethane and 1,4-diiodobutane) was performed in DMSO at room temperature and resulted in novel polymers with pyrrole and urazole linkages (Figure II.20d).

II.4.3 Copolymerization of bifunctional TAD-monomers

Most research on TAD compounds focuses on their ability to be used as very reactive dienophiles and/or enophiles.[12,83] This very pronounced reactivity immediately suggests opportunities for the formation of polymers in a stepwise manner via an AA-BB monomer approach with the aid of bisTAD molecules such as 4,4'-methylene-bis-(1,4-phenylene)-di-1,2,4-triazoline-3,5-dione (MDI-TAD, **9**, Figure II.21a).[135] Stadler *et al.* started research on this type of polymers in the early 1990's. Various bisTAD molecules were combined with respectively ditropyl (**10**),[136] bis(cyclohepta-2,4-6-trien-1-yl) sulfide (**11**)[137] and divalent dihydrophthalimides (such as **12**)[138] as useful bis-dienes (Figure II.21b).

The obtained polymers contained a rather rigid main-chain structure with M_n up to 18 000 g mol^{-1}. A Diels-Alder reaction under non-stoechiometric conditions offered the opportunity to prepare rigid rod telechelics with TAD end groups. In a later stage, these telechelics have been used as crosslinkers for polydienes (see subsection II.5.4).[139]

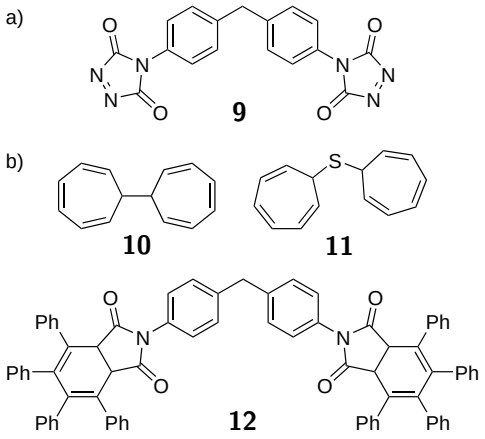


Figure II.21: a) divalent TAD, i.e. 4,4'-methylene-bis-(1,4-phenylene)-di-1,2,4-triazoline-3,5-dione (**9**); b) bis-dienes used for polymerisation via Diels-Alder, i.e. ditropyl (**10**), bis(cyclohepta-2,4-6-trien-1-yl) sulfide (**11**) and a divalent dihydrophthalimide (**12**).

Besides the combination of bifunctional TAD compounds with bis-dienes, other complementary partners are also suitable for polymerization. Indeed, while the work of Stadler *et al.* focused on consecutive Diels-Alder reactions, Alder-*ene* reactions have also been explored.[140] A very interesting example can be found in the work of Butler *et al.* in which bifunctional TAD molecules have been combined with styrene as a comonomer.[35] TAD can react with styrene in an initial slow Diels-Alder reaction, in which the aromaticity of styrene is lost. The resulting 1:1 adduct, however, undergoes a faster second reaction. Careful investigations showed that in this second reaction a 2:1 ratio is obtained for respectively the Diels-Alder adduct (**13**) and the Alder-*ene* (**14**) (Figure II.22). This cascade-type reaction of styrene with TAD has been used as a propagation mechanism in the copolymerization of styrene and (aromatic and aliphatic) bisTAD molecules, leading to polymers with molar masses up to 36 000 g mol^{-1} ($Đ = 3.34$).

Following their interesting results with styrene, the group of Butler studied the behavior of monofunctional TAD molecules in combination with a range of monofunctional components.[33,34,140,141] Those experiments resulted in a class of reactions, which showed that TAD undergoes an initial reaction with vinyl esters to generate (2+2) cycloadducts

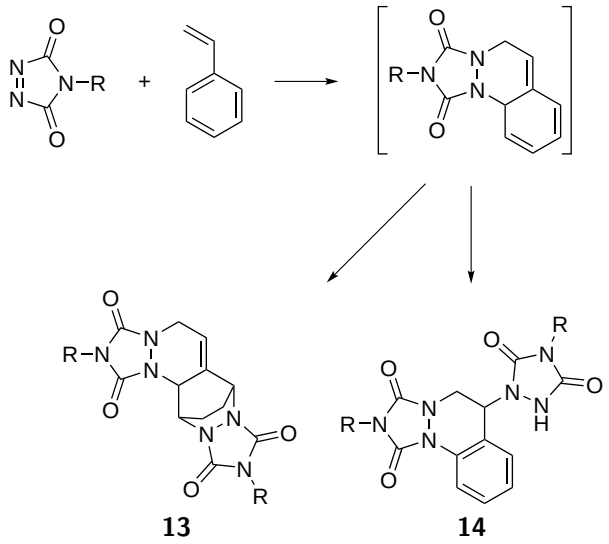


Figure II.22: Reaction between TAD and styrene: A first (Diels-Alder) reaction leads to a 1:1 adduct that is prone to a second TAD reaction (a 1:2 ratio was obtained for respectively the double Diels-Alder **13** and the Diels-Alder-ene adduct **14**).

(**15**) or rearranged derivatives thereof (**16**, Figure II.23a).[140,141] In an attempt to apply this reaction in polymer context, an adipate-based diester (**17**, Figure II.23b) was chosen (because of the small steric hindrance).[33] Low-molecular weight polymers were isolated via this route, most likely due to the degradation of the obtained polymers. An attempt was made to solve this degradation by replacing the starting monomer with di-isopropenyl adipate (**18**).[34] In practice however different fractions were obtained that were not well characterized, leading to the conclusion that vinyl esters are not ideal partners for TAD molecules due to the instability of the obtained adducts.

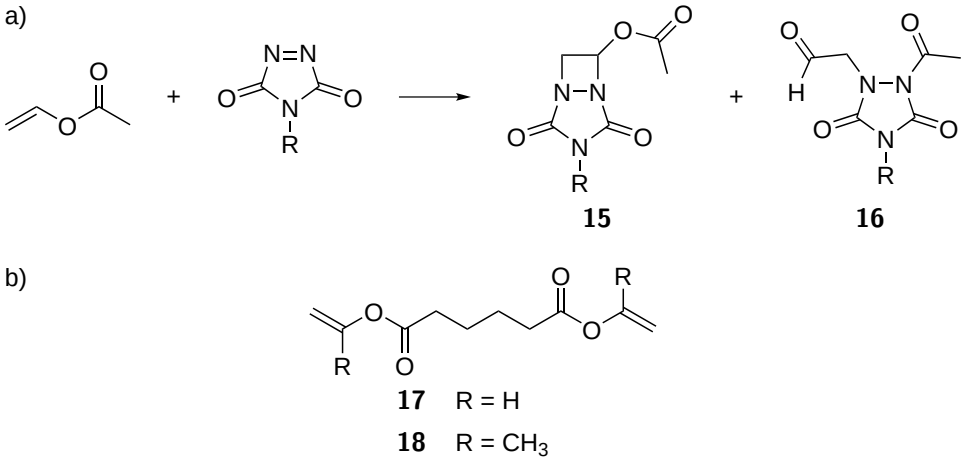


Figure II.23: a) Initial reaction between TAD and a vinyl ester leads to a mixture of (2+2) cycloadducts (**15**) and rearranged products thereof (**16**). b) Adipate-based diesters (**17** and **18**) used for polymer synthesis in combination with a bisTAD.

Inspired by the work of Butler, Williams *et al.* studied the reactivity of TAD molecules towards β-dicarbonyl components (see Figure II.24.[142] As these compounds were found to react with two TADs via two consecutive *ene* reactions on the enol (vinylogous carboxylic acid) form, they can be used as monomers in a copolymerization reaction with bisTAD compounds (**19**, **20**, **21** and **22**, Figure II.24).[75] Although the low MW model studies for this double ene reaction showed promising results, all acquired polymers had very low molar masses as a result of precipitation of low molecular weight oligomers in ethyl acetate.[142]

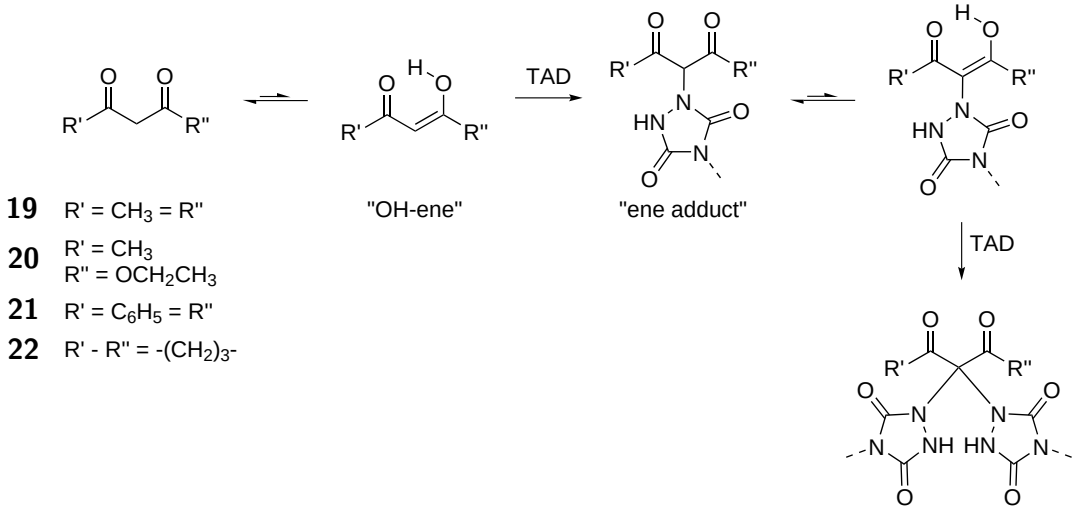


Figure II.24: β-dicarbonyl components can polymerize when combined with bisTADs via double *ene*-reaction on the enol form.

Starting from the original work of Butler with styrene (Figure II.22), Mallakpour *et al.* attempted in 1996 a step-growth polymerization of bivalent triazolinediones with 1,1-diphenylethylene.[143] The reaction was performed in DMF and proceeded very fast, i.e. 4 minutes compared to 30 minutes for styrene.[35] The structural and physical properties of the resulting polymers were studied and reported. A much slower reaction was obtained when the same bifunctional molecules were combined with *trans*-stilbene (reaction completed in several hours).[144] Mallakpour *et al.* continued this concept of tandem *ene* and Diels-Alder reactions by combining bisTAD moieties with (cheap) 1,4-cyclohexadiene.[145] In this case, an initial Alder-*ene* reaction leads to an intermediately formed 1,3-hexadiene. This diene is very reactive and will react *in situ* with another TAD molecule, leading to the formation of polymers chains. In order to make the obtained polymers more useful, Mallakpour and co-workers attempted to add flame-resistant properties by using trans-

3,3-dichloro-1-phenyl-1-propene.[146] The reaction at room temperature proceeded rather slowly (30 hours), but the reaction time could be reduced to 18 hours by refluxing the starting materials in DCM. Finally also optically active polymers (containing isoeugenol[147,148] and naphthalene[149] groups) were prepared via the same polymerization technique.

Apart from its application in polycondensations with monofunctional TAD compounds (see II.4.2), Mallakpour and Butler used N-methylpyrrole with a range of bifunctional TAD molecules (Figure II.25) to obtain polymers via a polyaddition approach.[150] Pyrroles can undergo two consecutive electrophilic aromatic substitution reactions which are extremely fast at room temperature (much faster than the substitution reaction at the pyrrole 3-position). The properties of the obtained polymers could be altered by varying the nature of the bifunctional TAD molecule. Similar results were obtained with other electron-rich aromatic compounds that have two reactive hydrogens, such as N,N,N',N'-tetramethyl-m-phenylenediamine.[151] This highly activated aromatic ring showed an exceptionally fast EAS reaction with TAD, affording the p-substituted aromatic polymer derivatives.

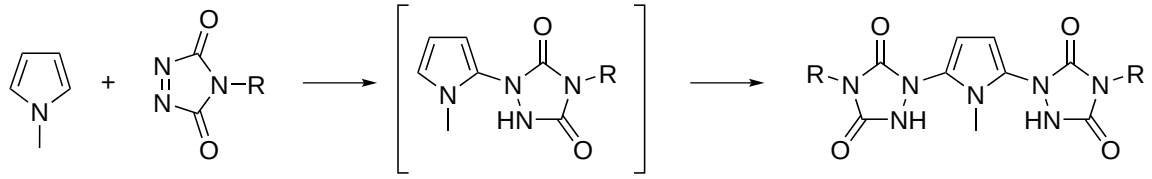


Figure II.25: Reaction between N-methylpyrrole and TAD selectively gives a 2,5-difunctionalized pyrrole.

II.4.4 Triazolinedione modification of polydienes

The major application of triazolinediones in polymer science, so far, lies in the low-temperature modification of polydienes, both in academic[82] and industrial context.[152–156] The alkene-TAD *ene* reaction is very versatile and gives an atom efficient and site selective way to functionalize substrates which are otherwise quite hard to chemically modify in a reliable way (Figure II.26).[64]

TAD-based modification of polydienes was introduced in the early 1970s by Saville[31] using natural rubbers, and was later on more extensively studied by Butler *et al.*[30,75] A wide range of polydienes (polybutadiene, polyisoprene, random styrene-butadiene copolymer

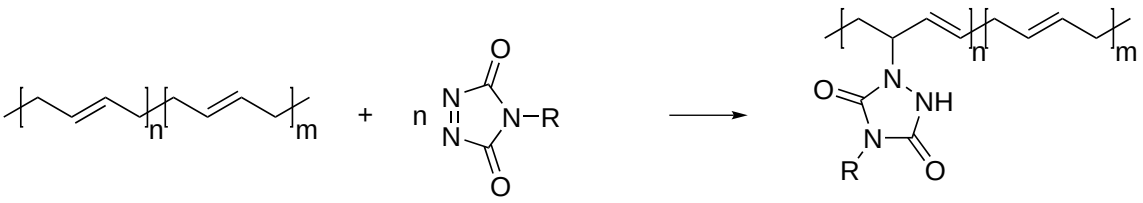


Figure II.26: Reaction between polybutadiene and TAD.

and a 1:1 alternating copolymer of furan and maleic anhydride) were modified with monofunctional TAD molecules. Although theoretically, it is possible to add 4 mole of reactant per repeating unit (i.e. 4 'active' C-H bonds), the solubility of the obtained polymers plays a significant role, and the subsequent reactions are usually kinetically disfavored. Polymers with modification degrees ranging from 5 till 100 % can be obtained, in which the polymers with lowest conversion demonstrated elasticity, which was an indication for possible secondary – supramolecular – crosslinking reactions (higher conversion led to rigid amorphous polymers with high Tg). However, follow-up studies[157,158] ruled out covalent crosslinking in these materials and supported the hypothesis that the highly polar pendant urazole groups have pronounced inter- and intramolecular hydrogen bonding interactions (Figure II.27a), which can be related to properties such as elasticity, changes in solubility character, thermal behavior and tensile strength. However, since this urazole-based association behavior is physical in nature, the polymer remains soluble. Stadler *et al.* studied this hydrogen bond network formation in detail using star-shaped polybutadienes.[159,160]

Many studies report a combination of polybutadiene (or co-polymers with isoprene[161,162]) with PhTAD. The profound influence of the hydrogen bonding on the properties of elastomers was extensively studied by a range of different characterization methods: on the solid material (or a solution thereof) (SEC[163], rheology[164,165], IR[166,167], birefringence[168], light scattering[169,170], DMA[171] and broadband dielectric spectroscopy[172]), in melt[173,174] and *in silico*.[175,176] Because the attached urazole groups contains a quite acidic proton (pKa $\approx$ 5), further modifications of this strong hydrogen bond donor are quite straightforward. Following a simple acylation reaction that 'caps' this acidic N-H group, polymers with a wider range of properties can be obtained by using different acid chloride modifiers, while the supramolecular self-associating behaviour is also blocked.[177] The degree of modification can be easily controlled by the amount of PhTAD, and the type of modification can be controlled by the choice of acylating group (Figure II.27c). In this way, also optically active acid chlorides can be introduced on polydienes.[178]

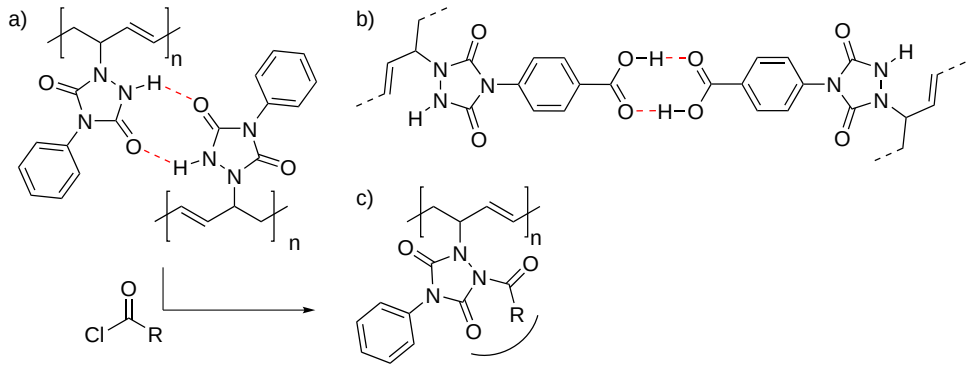


Figure II.27: a) Secondary crosslinking of a polybutadiene functionalized with PhTAD and b) extra secondary crosslinking of polybutadiene functionalized with a 4-carboxy-derivative of PhTAD. c) The acidic urazole proton can be (partially) acylated, thereby blocking the self-associating behavior.

Extending upon the concept of urazole-based hydrogen bonding networks (cf. Figure II.27a), Stadler *et al.* explored a range of TAD reagents that would enhance this supramolecular behavior, including hydroxyl-, nitro- and carboxyl- functional TAD molecules.[179] It was shown that the addition of a hydroxyl group as an extra hydrogen bond donor resulted in the formation of extended 'junction zones' instead of point-like linkages. The use of a nitro-functionalized TAD moiety showed a similar hydrogen bonding behavior as the original PhTAD complexes, and were used to convert the polybutadienes into ionomers.[180] The modification of polydienes with 4-carboxy-derivative of PhTAD (PhTAD-COOH) was first described in patent literature[181–183] and later Hilger *et al.* studied the hydrogen-bonding behavior in these materials, which was found to be similar to that of a covalently crosslinked material up to 80 °C.[184] The mechanical properties were superior to those of PhTAD-based H-bonded networks, and actually comparable to thermoplastic elastomers of the covalent multiblock copolymers type, with high modulus and tensile strength.[63,185–190]

The PhTAD-COOH functionalized 'H-bond crosslinked' polydienes (Figure II.27b) have been studied quite extensively as materials. Although experiments were conducted to determine the best position of the carboxyl group on the aromatic system, the original para positioned group gives the most satisfactory results as proven by DSC, birefringence, DMA, stress-strain experiments, SAXS, crystallography and deuterated NMR.[186] In addition to simple polybutadienes , different matrices have also been successfully modified into networks using PhTAD-COOH,[191] including copolymers of butadiene and isoprene,[192], oligo-[193] and polyisobutylene,[194] and polystyrene-polybutadiene block copoly-

mers.[195] Similar results have been obtained using a range of PhTAD-COOH derivatives, such as 5-isopthalic acid,[196–198] benzamide[199] or phenylazobenzoic acid.[199]

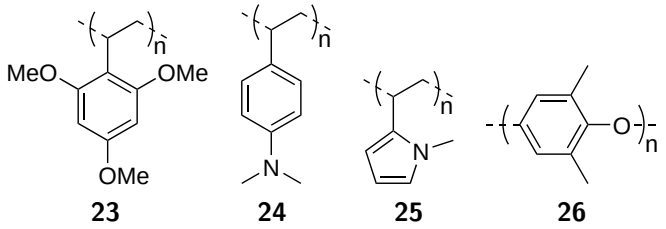
Not only modification with monofunctional components was attempted but also bisTAD molecules were reacted with the aforementioned polydienes, resulting in highly crosslinked polymer networks.[30,200] De Lucca Freitas and Stadler studied the characteristics of the network formation in the reaction of polybutadiene and MDI-TAD (**9**, Figure II.21). This reaction was studied in solution as a model system, which allowed monitoring of the crosslinking kinetics, in function of the gelation process[201,202] and the primary molecular weight of the polymer matrix.[203] In later studies, a variety of crosslinkers (containing azo-dyes[204] or polyethyleneglycol chains[173,175]) and matrices[155,156] were used to obtain different network properties.

Given the extremely reactive nature of TAD towards enes, the crosslinking kinetics of polydienes is often hard to control in order to achieve homogeneous material properties, especially under solvent-free bulk conditions. For this, in line with the concept of blocked isocyanates, attempts have been made to temporarily 'block' the TAD functionality in order to release it at a higher temperature, giving a controlled crosslinking reaction. Green *et al.* have shown that adamantylideneadamantane (**8**, Figure II.15), which reacts with TAD at room temperature to give a (2+2) cyclo-adduct,[120,205] can undergo the reversed reaction above 70 °C, which gives a thermally triggered TAD generating reaction. This 'blocked TAD' approach was used for the controlled crosslinking of polydienes in bulk (without solvent). Heating of a mixture of polydiene and a blocked bisTAD reagent at 100 −120 °C in a vacuum oven, gives a homogeneous network formation.[206]

Very recently, Zhao *et al.* introduced triazolinediones as an efficient post-polymerization tool for ring opening metathesis polymerization (ROMP) derived polymers. The TAD based Alder-*ene* reaction was used to efficiently post-functionalize well-defined ROMP polymers. Both monofunctional and bifunctional TAD molecules were employed to functionalize and crosslink poly(N-propyl-5-norbornene-exo-2,3-dicarboximide).[207]

II.4.5 Crosslinking and functionalization of other polymer matrices with TAD reagents

Next to alkenes, electron rich aromatic rings can also be included in simple linear polymers as reaction partners for TAD. On the one hand, vinyl-type (co)monomers, having an activated aromatic ring, can be easily synthesized and incorporated into linear polymer chains (Figure II.28), which can then be functionalized or crosslinked with suitable TAD reagents.[208] Poly(2,4,6-trimethoxystyrene) (PTMS, **23**) reacts only very slowly with PhTAD (10 days at room temperature, two days in boiling DCM) while the reactions of poly(4-(N,N-dimethylamino)styrene) (PDMAS, **24**) and poly(N-methyl-2-vinyl-pyrrole) (PMVP, **25**) were much more rapid. For the latter two materials, the TAD functionalization reactions proceed fast at room temperature and resulted in respectively 90 and 97% incorporation of PhTAD. Stock *et al.* found that PhTAD-functionalized PDMAS (and related copolymers) show a weak hydrogen bond between the urazole proton and the DMA-group.[209]



23 **24** **25** **26**

Figure II.28: Synthesized polymers for modification with TAD via EAS: poly(2,4,6-trimethoxystyrene) (**23**), poly[4-(N,N-dimethylamino)styrene] (**24**), poly(N-methyl-2-vinyl-pyrrole) (**25**) and poly(oxy-2,6-dimethyl-1,4-phenylene) (**26**).

On the other hand, a whole range of new polymer blends containing poly(oxy-2,6-dimethyl-1,4-phenylene) (PPE, **26**, Figure II.28) were investigated for different applications at the end of the 1980's. As the repeating unit in these materials is actually a rather activated aromatic ring, these polymers can be directly modified using the EAS reaction with TAD. Indeed, Stadler *et al.* showed that PPE can be functionalized with TAD compounds at room temperature.[210,211] By addition of the highly polar urazole groups, physical linkages to other polymers and improved adhesion were achieved. With the goal to modify commercial blends, mixtures of polystyrene (PS) and PPE were functionalized with TAD to various extent. It was shown that PS and PPE, modified with up to 10% PhTAD, were still miscible, making this a valuable synthesis route for the modification of PPE blends.

II.4.6 Surface modification

Shortly after the first publications concerning TAD chemistry with unsaturated polydienes, investigations started on polymer surface modification. Cutts *et al.* has been treating surfaces of elastomers with TAD components (both mono- and bifunctional).[212] In this way not only the adhesion could be improved, but also resistance to peeling with flexible paints improved while the surface tack of the elastomers reduced. This proved to be an advantageous method over the prior art (chlorination and halogen donor techniques), particularly because the applied triazolinediones are relatively mild and non-corrosive.

Triazolinedione chemistry could also be used to verify the successful incorporation of diene (*ene*) moieties in polymers. In this context, Gleason *et al.* reacted PhTAD on a poly(furfuryl methacrylate) (PFMA) film.[213] This PFMA film is actually a furan-ring functionalized solid surface, achieved by chemical vapor deposition. By analysis of the FT-IR and XPS spectra, obtained before and after the reaction with PhTAD, proof of the successful modification of the furan moiety was given.

II.4.7 Other uses of triazolinediones in macromolecular context

Besides the already mentioned categories, there are also some isolated publications involving triazolinediones in polymer science. One of these examples is the synthesis of macrocyclizations described by Schumann *et al.*[214] In this work, the authors use a combination of bifunctional TAD and sorbic acid (or sorbyl derived) molecules in high dilution. The formation of the macrocycles consists of two steps: first an intermolecular Diels-Alder reaction of the bifunctional diene with bisTAD, followed by an intramolecular ring closure (Scheme 40). Via this procedure, larger cycles up to 21-membered rings could be obtained.

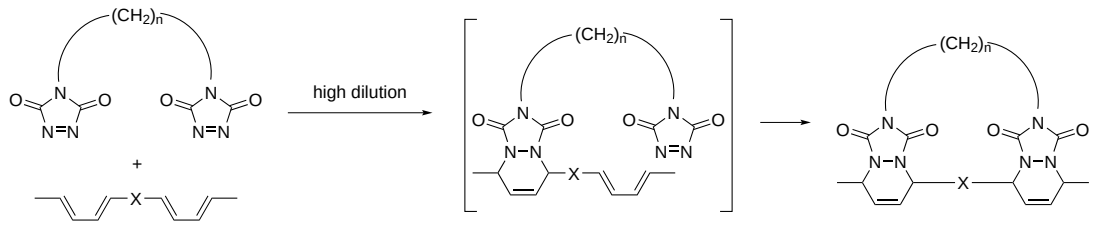


Figure II.29: Formation of macrocycles by combining bisTAD molecules (n=2 or 6) with a bis-diene.

Very recently, Craig and co-workers came with another use of TAD molecules, more specifically in the area of mechanochemistry.[215] In this work, embedding mechanophores into a poly(dimethylsiloxane) (PDMS) network, allowed for covalent bond activation under mechanical stress. This has been shown by applying a mechanical force onto a PDMS containing PhTAD-anthracene crosslinks. When sufficient stress was applied, the retro-Diels-Alder reaction occurred, leading to the release of PhTAD and unveiling the fluorescent behavior of the anthracene moiety (Scheme 41).

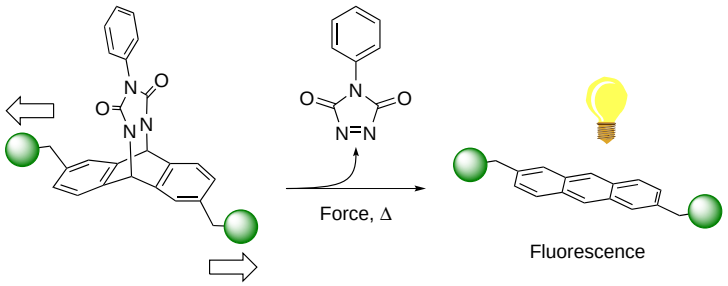


Figure II.30: Release of PhTAD (**1**) by mechanochemistry.[215]

II.5 The use of triazolinediones in *click-like* applications

The concept of '*click* chemistry' was introduced in 2001[216,217] and has made a huge impact on the entire scientific community,[218] despite the fact that *click* chemistry is often just focused on repurposing long-known reactions for new applications. The simple but visionary act to define a set of characteristics that a synthetic bond-forming reaction should ideally meet, offers a highly subjective but at the same time quite useful conceptual framework to consider chemical reactivity in terms of the possible applications a reaction may have. In short, the introduction of *click* chemistry was a strong encouragement for chemists to focus on reactions that work in almost any context and are user-friendly to the point where also non-chemists can benefit from the power of chemical synthesis.[218]

The list of *click-criteria* are now regarded as the minimum set that 'new' *click* reactions should have.[219] The initial introduction of the *click* chemistry concept was tailored for the example of the development of new drugs, but – as anticipated in the original report – has also been adopted outside this specific area of human endeavour. For biology oriented

applications, compatibility with water and physiological stability of adducts have been identified as additional desirable characteristics. For other areas of research that were quick to adopt *click* chemistry principles, such as polymer science, the concepts and criteria have also been customized and expanded to fit the largest possible number of applications.[220]

Although hetero-Diels-Alder reactions have been called 'beautiful representatives of *click* chemistry ideals' by Sharpless and Finn, TAD-based chemistry has not been discussed in the context of *click* chemistry for almost a decade after the initial report.[110] Indeed, apart from having a reputation as the 'most reactive' dienophiles, TAD compounds also have a reputation of being 'exotic' reagents and have been generally regarded as highly unstable species. Nevertheless, as is shown in the previous sections, the synthesis of TAD reagents can be straightforward and mostly involves steps that are high yielding and do not require a purification step, at least when chemoselectivity issues are carefully considered in the choice of starting materials and reagents.

Although many of the examples discussed in sections II.3 and II.4 can in some way or another be considered as *click* reactions, those sections mainly focus on well-known simple TAD compounds that have no other functional moieties, and provide a good illustration of the reactivity of TADs, but do not readily showcase the potential of triazolinediones in *click* chemistry, because of the often highly specific (i.e. non-modular) nature of the application. Here an overview of applications of TAD reagents is given, that are closer to what is generally expected of truly modular *click* chemistry tools.

II.5.1 *Click*-bioconjugation of peptides and proteins

Barbas *et al.* were the first to explicitly report on TAD reagents as useful tools for *click* chemistry, in their 2010 paper on a *click-like* conjugation strategy for natural peptide and protein substrates. Herein, Barbas explored the use of triazolinediones as an efficient tyrosine bioconjugation strategy.[110,118] Although TAD molecules are known to react slowly with phenol substrates via an EAS pathway (see subsection II.3.3), Barbas serendipitously observed that this reaction is greatly accelerated in aqueous medium. Conversely, similar EAS or *ene*-type reaction with tryptophane side chains (indole core) are not accelerated, offering a way to site-selectively label tyrosine residues in natural peptide and protein

substrates (Scheme 42).

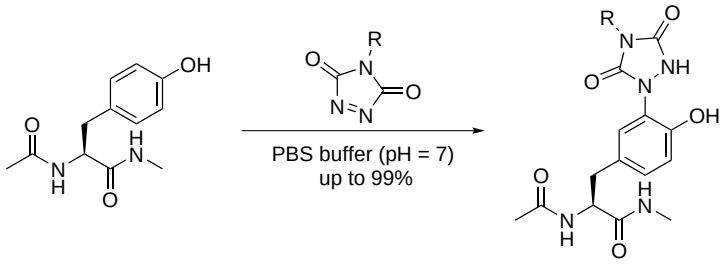


Figure II.31: Model reaction of the tyrosine bioconjugation (R = Ph or Me).

Following the initial report, Barbas and co-workers showed the true potential of this tyrosine bioconjugation with a range of additional experiments and a systematic study of TAD orthogonality towards different amino acid side chains.[111] For this, they used a synthetic decapeptide with unprotected side chains, containing all of the potentially reactive amino acids Trp, Ser, Glu, Lys, Arg, and His, as a stringent chemoselectivity test. Even when three equivalents of a TAD reagent were applied, this peptide was site-selectively functionalized at the tyrosine residue. As reactions with primary amines are typically hard to avoid (see subsection II.3.5), the success of this *click-like* tyrosine conjugation method can also be explained by the use of a buffered medium (pH 7 phosphate buffer), which will keep most of the free amines (on the lysine and N-terminal residue) in their unreactive protonated form. Purification by using reversed-phase HPLC yielded pure labeled peptides in approximately 60% yield.

To expand this tyrosine-selective *click*-modification even further, Barbas' group also implemented it for site-selective and orthogonal protein multifunctionalizations.[111] In this case, orthogonal trifunctionalization at tyrosine, cysteine, and lysine residues of bovine serum albumin (BSA) and human serum albumin (HSA) was achieved. Cysteine and lysine were modified with a fluorescein maleimide and 11-(dansylamino) undecanoic acid, respectively, while the tyrosine units were successfully reacted with TAD derivatives.

In a related study by Barbas and co-workers,[111] pegylation of proteins was studied, which is one of the most important protein conjugation reactions for pharmaceutical applications. For this, a 5 kDa polyethylene glycol chain with a TAD end group was prepared through CuAAC reaction by using an azide-functionalized urazole (Figure II.32). After oxidation, the macromolecular TAD reagent was directly coupled to chymotrypsinogen A

(which contains four tyrosine units). Reactions were performed in buffer solutions (pH 7.4) with 10 equivalents of the PEG-TAD compound. After removal of the excess of PEG, predominant formation of mono-PEG addition products was observed, pointing towards a site-selective modification.

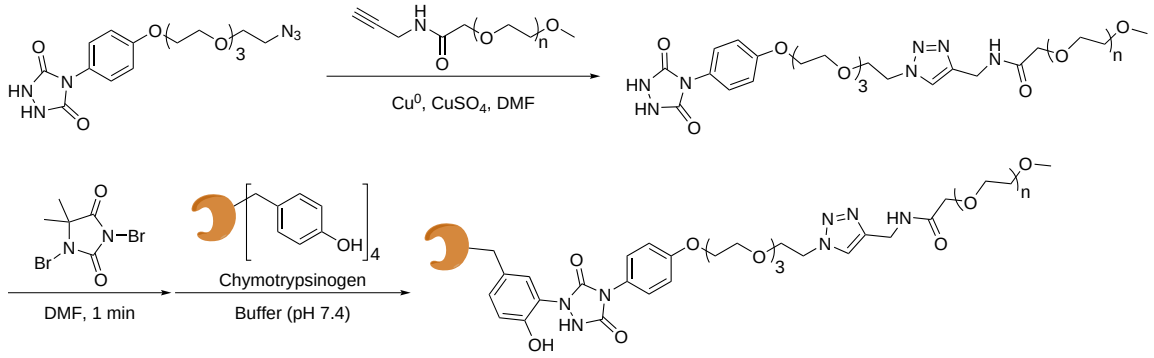


Figure II.32: Treatment of a buffered aqueous solution of chymotrypsinogen with an excess (10 equivalents) of a freshly oxidized solution of PEG-TAD in DMF leads to selective PEGylation of the protein substrate.

In another biomedical application, Barbas and co-workers used the TAD-tyrosine *click* conjugation to couple the anti-HIV drug aplaviroc with a monoclonal antibody.[111] The targeted delivery of drugs through antibody-drug conjugates can be of use for many therapeutic applications. As a model monoclonal antibody, the well-characterized trastuzumab was used. An alkyne-containing derivative of aplaviroc was coupled to an azido-urazole by using a CuAAC reaction. This precursor was oxidized to yield the TAD derivative, which was immediately used for labeling of trastuzumab. Again, the obtained protein mostly showed the incorporation of a single drug molecule. This drug-antibody conjugate was further shown to retain both its antiviral as well as its antigen binding activity.

Bauer *et al.* used the tyrosine-TAD click reaction to prepare DNA-protein conjugates, making use of a range of interesting heterobifunctional cross-linkers (Figure II.33).[221] These bifunctional cross-linkers incorporate a TAD moiety to ensure the fast and selective reaction with tyrosines, and another functionality to couple a DNA strand. For this last coupling, a variety of different *click* reactions was tested, i.e. maleimide-thiol Michael addition (**27**-**28**), CuAAC reaction (**29**), and strain-promoted copper-free AAC reaction (**30**). All three orthogonal *click* strategies gave conjugates that outperformed those obtained through more classical lysine-based protein ligation.

In a recent paper from a pharmaceutical industry group, Hu *et al.* report the use of

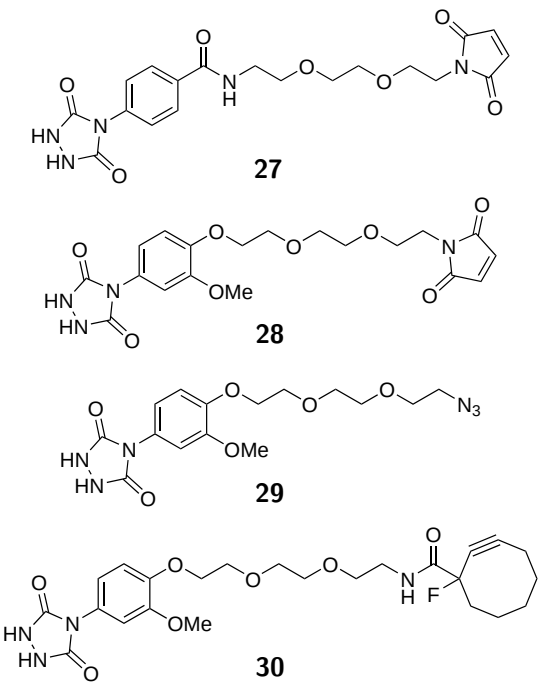


Figure II.33: Urazole precursors (**27**, **28**, **29** and **30**)for TAD-based heterobifunctional cross-linkers useful in sequential orthogonal click reactions.

the tyrosine-TAD *click* reaction to tackle a challenging protein substrate of considerable pharmaceutical interest.[127] CRM197 is a nontoxic mutant diphtheria toxin that has been extensively used as the protein carrier in many licensed vaccines and has well-proven safety and efficacy. As this substrate apparently has some very reactive lysine residues that compete for the usually more reactive tyrosine side chains, the group undertook a systematic study of TAD site-selectivity for several proteins, and examined the influence of reaction conditions. They found the use of an amine-based buffer to be helpful to suppress lysine conjugation, as it may also act as a scavenger for isocyanates. They also found that the amount of TAD-reagent has to be varied in the range of 1.1 to 30 equivalents, depending on the substrate, to achieve a complete conjugation, reflecting the varying 'availability' of tyrosine residues in different proteins.

As can be expected, a clear correlation was observed between the labeling efficiency of tyrosine residues and their relative exposure to the protein surface in the X-ray structure. Similarly, for CRM197, which forms homodimers in solution, the tyrosine residues involved in the dimerisation were found to be unreactive towards TAD. When CRM197 is treated with an excess of TAD linker, a reproducible labeling of the same four specific tyrosine residues was observed, out of a possible eighteen. Using an alkyne-functional TAD reagent,

a well-defined glycosylated CRM197 conjugate was obtained through sequential tyrosine-TAD click and CuAAC reactions. Such glycoconjugates can be used as vaccines, and the overall strategy seems applicable for the synthesis of many well-defined protein conjugates.

In a follow-up study, the tyrosine click bioconjugation strategy was further developed towards the preparation of glycoconjugates.[222] Here, a heterobifunctional TAD-azide linker was first site-selectively *clicked* to a protein substrate, followed by a copper-free strain-promoted cycloaddition to cyclooctyne modified carbohydrates. The synthesis was employed for vaccine antigens and was found to be very versatile and high yielding.[223]

Madder and co-workers developed an alternative TAD-based strategy to functionalize peptides prepared through classical solid phase synthesis.[224] Instead of relying on tyrosines, which react very sluggishly with TADs in non-aqueous media, a highly reactive furan residue was incorporated in a synthetic peptide, using the unnatural but commercially available amino acid furylalanine. A model peptide featuring a furylalanine was synthesized on a solid support. As a classical maleimide-furan Diels-Alder conjugation proved to be not very efficient, the more reactive triazolinediones were investigated. Indeed, by using only 3 equivalents of PhTAD in dichloromethane, a complete reaction was observed with the solid supported peptide in only 15 minutes at room temperature. However, detailed investigations showed that the expected Diels-Alder adduct was not observed, but instead the product of a clean furan-PhTAD electrophilic aromatic substitution (EAS) was found. The reaction proved to be a useful tool for quantitative and site-selective peptide labeling, and was also used in a simple orthogonal peptide labeling protocol. In this case, a simple FRET-probe could be synthesized using the commercially available TAD based compound DMEQ-TAD.[224]

II.5.2 *Click*-derivatisation of low-abundant lipid metabolites in biological samples

The quantification of lipid metabolites can be of great diagnostic value, but standard chromatographic and mass spectrometric methods are hampered by high detection limits. Yamada *et al.* reported in 1990 that triazolinediones are ideal reagents to derivatize conjugated dienes in natural lipids, even in complex and dilute biological samples. Yamada synthesized the now commercially available fluorescent DMEQ-TAD reagent, and used it to assay and quantify various hormonally active and important vitamin D metabolites.[58] The TAD-diene hetero Diels-Alder *click* reaction is so fast and orthogonal that vitamin D metabolites, at a concentration as low as 10^{-8} M, could be reliably labeled and then quantified via HPLC with a fluorescence detector.[225] This technique has a detection limit for vitamin D-related metabolites down to 0.1 fmol. In a similar application, Shimada *et al.* employed fluorescent TADs as a derivatization tool to quantify 7-dehydrochlolesterol, a steroid that also incorporates a diene.[40]

In later work, the determination of vitamin D metabolites with the aid of TADs was extensively studied (especially in mass spectrometry), and is a standard method in the field, reflected by the fact that DMEQ-TAD is commercially available.[226–229] In 2005, Murao *et al.* showed the unique combination TAD as a powerful dienophile with an efficiently ionizable functionality (ferrocene group).[230] The TAD-ferrocene reagent was found to give the most sensitive detection method for vitamin D-like metabolites.

II.5.3 Triazolinediones as tools in modular chemical library synthesis

For the parallel synthesis of structurally diverse libraries of chemical compounds, it is important that each reaction step proceeds with the maximum efficiency, and that reaction work-up and purification can be standardized to a point that allows for automated synthesis and purification steps. *Click* chemistry concepts are thus ideally suited for these needs. In practice, however, the number of truly diversity-generating *click* reactions is limited, and it is hard to build libraries using only *click* reactions.

The famous Diels-Alder reaction, which is arguably the most powerful construction reaction in organic synthesis, has a few drawbacks with regard to *click* chemistry ideals despite the fact that it has been denoted earlier as a *click* reaction.[231] Although most Diels-Alder reactions have a high intrinsic driving force, obtained yields and conversions are often limited because of kinetic reasons, as they require considerable thermal activation. The most common and reliable way to achieve good yields in reasonable time frames for Diels-Alder reactions, is the use of an excess of one of the reaction partners. However, this straightforward strategy typically requires a chromatographic separation of the desired cycloadduct from the excess of diene or dienophile as an 'offensive byproduct'. This chromatographic separation can be avoided if the excess diene or dienophile can be selectively derivatized with a secondary Diels-Alder reaction that does meet *click* chemistry ideals, if this gives non-offensive cycloadducts that can be easily removed from reaction mixtures.

In 2003, Curran *et al.* introduced the use of triazolinedione reagents in the field of fluorous tagging.[232] Their work in fluorous tagging focused on suitable diene scavengers. A range of highly fluorinated dienophiles including maleimides triazolinediones was explored. These fluorous tags were used to treat Diels-Alder reaction mixtures containing an excess of diene as the only 'side product', which would allow for removal of the initially non-reacted diene by a simple solid phase extraction (Figure II.34). As expected, the TAD-based fluorous tags outperformed their maleimide counterparts. Furthermore, because of the characteristic colour of TADs, a highly practical work-up protocol was developed in which a simple titration of the reaction mixture with the TAD-scavenger was performed, until the persistence of a pink colour was observed.

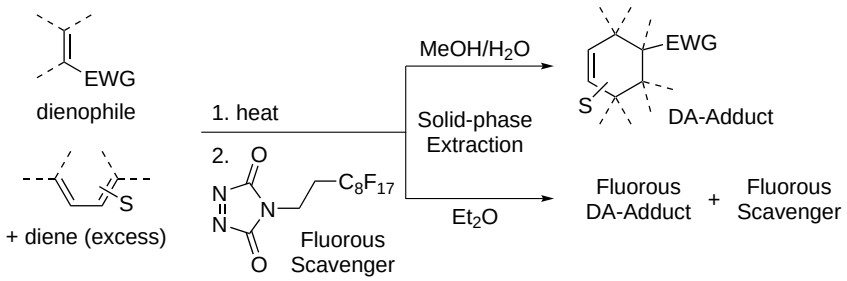


Figure II.34: Fluorous tagging of excess dienes in reaction mixtures facilitates purification of a 'generic' Diels-Alder adduct.[232]

In a report by a pharmaceutical industry group, Pieken *et al.* describe the use of a TAD-based solid phase diene scavenger to separate reaction products from reagents in the

solution phase synthesis of oligonucleotides.[233] By using a diene-functionalized protecting group during oligonucleotide synthesis, all compounds that incorporate this protecting group can be selectively removed from complex reaction mixtures and excess reagents with a TAD-resin loaded column (Figure II.35). Using standard deprotection conditions, the desired compounds can then be eluted from this column, and oligonucleotide products can be obtained in pure form without the need for complicated chromatography. A simple TAD-functionalized scavenger resin was obtained by treating a diene-functionalized polystyrene column with a large excess of a bifunctional TAD reagent. For this, a sterically constrained bisTAD compound was chosen to minimize intra-resin crosslinking. This strategy seems applicable for the temporary and highly selective immobilization of a wide range of products onto a solid phase, as long as suitable diene-functionalized protecting groups can be introduced into the substrate. A range of dienophiles was explored, but triazolinediones again emerged as the reagents of choice for this catch-and-release strategy.

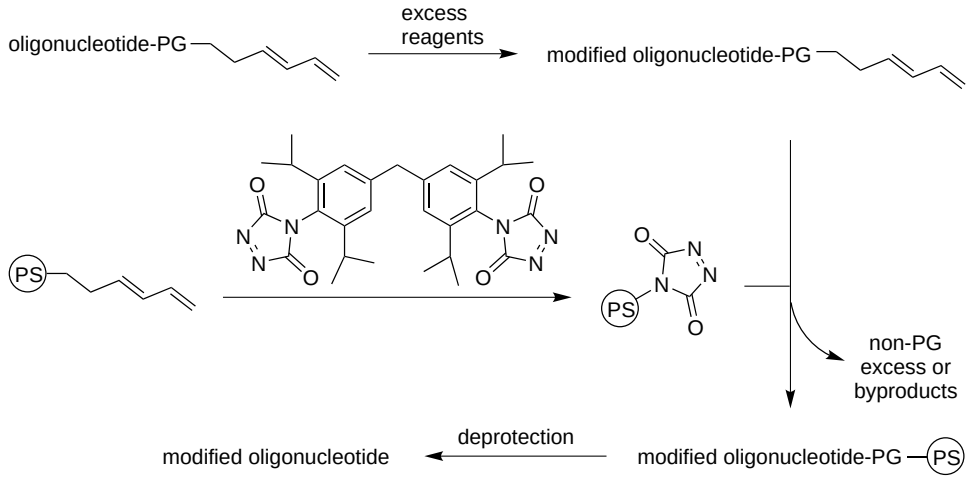


Figure II.35: A Diels-Alder catch-and-release strategy for the solution phase synthesis of oligonucleotide substrates, based on diene-functionalized protecting groups and TAD-functionalized resins.

Besides its use as a *click-like* clean-up tool for reaction mixtures, triazolinediones can also be used directly in the synthesis of libraries of diverse screening compounds. Porco and co-workers were the first to describe the systematic use of variously functionalized triazolinedione building blocks in a stereocontrolled synthesis of a complex library.[234] Similarly, Schreiber *et al.* developed a highly efficient protocol based on eneyne metathesis of simple olefin building blocks, resulting in 1,3 dienes that are then 'rigidified' into a range of diverse polycyclic scaffolds.[235]

II.5.4 *Clicking* and *unclicking* protecting groups in organic synthesis

TAD reagents can be used as protecting groups for dienes,[236] but the deprotection requires a hydrolysis in strongly alkaline medium at elevated temperatures. Thus, as is often the case in implementing protecting groups in organic synthesis, this strategy adds a lot of practical difficulties to a synthesis, making it a less efficient process. In ideal synthetic routes, the use of protecting groups should be completely avoided.[237] However, protecting groups than can be installed and removed in a straightforward way, with regard to the *click* chemistry criteria, could be considered as 'ideal' protecting groups.

In 2003, Baran *et al.* implemented triazolinediones as such a highly efficient protecting group for the N-H and 2,3 π-bond of indoles.[76] During their total synthesis of Okaramine N, a nucleophilic indole needed to be protected during an oxidation step of another (less reactive) indole. Inspired by the well-known similarity in reactivity between singlet oxygen and triazolinediones, the commercially available methyl substituted TAD (MeTAD) was first reacted with this bis-indole. This gave a highly selective, atom economic and fast (10 min) reaction with MeTAD in dichloromethane at $-5\,^{\circ}\mathrm{C}$ to form exclusively the ene product at *C3* of the N-unsubstituted indole subunit (Figure II.13).[76,238] Simple evaporation gave the product that could be used in the oxidation step. The purified oxidation product could be cleanly converted to the free indole by simply heating the neat material *in vacuo*, which removed volatile MeTAD and directly gave the deprotected product.

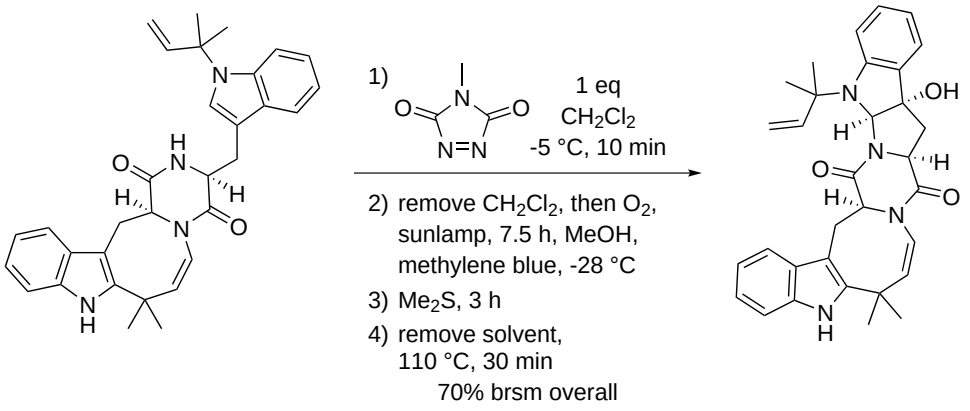


Figure II.36: Use of MeTAD as a volatile *click*/*unclick* protecting group in the final steps of the total synthesis of okaramine N.

II.6 Conclusions and perspectives

Apart from having a reputation as being a very reactive species that can participate in a whole range of reactions with specific classes of olefin-type substrates, triazolinediones compounds also have a reputation of being 'exotic' and unstable reagents. This chapter tried to change that perspective by serving as an introductory guide to the exceptional *click-like* reactivity of 4-substituted triazolinediones.

In the first part of this chapter (II.2) it is shown that the synthesis of TAD reagents can be straightforward and mostly involves steps that are high yielding and in most cases do not require a purification step. A survey of known practical synthesis methods for TAD compounds revealed that these almost invariably involve the oxidation of the corresponding 1,2,4-triazolidine-3,5-dione ('urazole'). For the assembly of these heterocyclic precursor urazoles, a large variety of methods is now available, using a number of simple starting materials. Likewise, many different oxidation methods for urazoles have been explored, with varying degrees of efficiency and chemoselectivity. Therefore an informative overview of urazole syntheses and of urazole oxidation methods (section II.2) is given. In the three last sections of this chapter (II.3-II.5), an instructive overview of the reactivity of triazolinediones, illustrated by various applications, is provided. While TADs are highly activated species and can actually participate in a large variety of reactions, depending on conditions and reaction partners, only a brief discussion of the most important reaction partners and possible side reactions (for this work) was provided herein (section II.3). In the next sections, two distinct case studies of TAD reactivity in practical applications were presented. A first case study was a comprehensive overview of the use of triazolinediones in the field of polymer science (section II.4), while a second case study discussed the use of these reagents in *click-like* chemistry applications (section II.5). This final section is of great importance to give a good benchmark for this doctoral work.

II.7 Bibliography

[1] H. Freeman, *Aatcc Review* **2004**, *4*, 16–21.

[2] J. M. Stellman, *Encyclopaedia of Occupational Health and Safety, Vol. 4*, International Labour Organization, **1998**.

[3] F. M. Lewis, M. S. Matheson, *Journal of the American Chemical Society* **1949**, *71*, 747–748.

[4] K. Golka, S. Kopps, Z. W. Myslak, *Toxicology Letters* **2004**, *151*, 203–210.

[5] A. Puntener, J. Fennen, C. T. Page, *Journal - Society of Leather Technologists and Chemists* **1996**, *80*, 1.

[6] G. Moad, D. H. Solomon in *The Chemistry of Radical Polymerization* (Eds.: G. Moad, D. H. Solomon), Elsevier Science Ltd„ Amsterdam, 2nd ed., **2005**, pp. 49–166.

[7] J. S. Park, K. Takahashi, Z. Guo, Y. Wang, E. Bolanos, C. Hamann-Schaffner, E. Murphy, F. Wudl, H. T. Hahn, *Journal of Composite Materials* **2008**, *42*, 2869–2881.

[8] M.-H. V. Huynh, M. A. Hiskey, E. L. Hartline, D. P. Montoya, R. Gilardi, *Angewandte Chemie International Edition* **2004**, *43*, 4924–4928.

[9] A. Jacobs, *Understanding Organic Reaction Mechanisms*, Cambridge University Press, **1997**.

[10] R. Cookson, S. Gilani, I. Stevens, *Journal of the Chemical SocietyC-Organic* **1967**, 1905.

[11] J. Thiele, O. Stange, *Justus Liebigs Annalen der Chemie* **1894**, *283*, 1–46.

[12] S. Radl in *1,2,4-Triazoline-3,5-Diones, Vol. 67*, Elsevier, San Diego, **1997**.

[13] R. A. Novikov, V. A. Korolev, Y. V. Tomilov, *Russian Chemical Bulletin* **2011**, *60*, 1685–1693.

[14] A. Holleman, E. Wiberg, N. Wiberg, *Inorganic Chemistry*, Academic Press, **2001**.

[15] K. De Bruycker, S. Billiet, H. Houck, S. Chattopadhyay, J. Winne, F. Du Prez, *Chemical Reviews* **2015**.

[16] G. Pellizzari, G. Cuneo, *Gazzetta chimica Italiana* **1894**, *24*, 499.

[17] L. F. Audrieth, E. B. Mohr, E. Davis, W. R. Tomlinson in *Urazine*, John Wiley and Sonc, Inc, **1953**, pp. 29–32.

[18] H. Giesecke, R. Merten, L. Rottmaier, *EP0044421* **1982**, Di– and oligo–1,2,4–triazolidine–3,5 diones and processes for their preparation.

[19] H. Giesecke, M. Henning, L. Rottmaier, *US4386213* **1983**, Di– and Oligo–1,2,4–triazolidine–3,5–diones and processes for their production.

[20] H. Giesecke, M. Henning, L. Rottmaier, *US4429135* **1984**, Process for the production of 1,2,4–triazolidine–3,5–diones monosubstituted in the 4–position.

[21] R. Sowerby, *WO8707892* **1987**, Urazoles compositions useful as additives for functional fluids.

[22] G. Zinner, W. Deucker, *Archiv der Pharmazie* **1961**, *294*, 370–372.

[23] R. Cookson, S. Gupte, I. Stevens, C. Watts, *Organic Syntheses* **1971**, *51*, 121.

[24] G. W. Breton, M. Turlington, *Tetrahedron Letters* **2014**, *55*, 4661–4663.

[25] S. Ogawa, S. Ooki, M. Morohashi, K. Yamagata, T. Higashi, *Rapid Communications in Mass Spectrometry* **2013**, *27*, 2453–2460.

[26] R. C. Cookson, S. S. H. Gilani, I. D. R. Stevens, *Tetrahedron Letters* **1962**, *3*, 615–618.

[27] K. Shimada, T. Oe, T. Mizuguchi, *Analyst* **1991**, *116*, 1393–1397.

[28] J. C. Stickler, W. H. Pirkle, *The Journal of Organic Chemistry* **1966**, *31*, 3444–3445.

[29] B. T. Gillis, R. A. Izydore, *The Journal of Organic Chemistry* **1969**, *34*, 3181–3187.

[30] G. B. Butler, A. G. Williams, *Journal of Polymer Science: Polymer Chemistry Edition* **1979**, *17*, 1117–1128.

[31] B. Saville, *Journal of the Chemical Society D: Chemical Communications* **1971**, 635–636.

[32] K. Wald, H. Wamhoff, *Chemische Berichte* **1978**, *111*, 3519–3523.

[33] K. B. Wagener, K. A. Matyasjewski, G. B. Butler, *Journal of Polymer Science: Polymer Letters Edition* **1979**, *17*, 129–137.

[34] K. A. Matyjaszewski, K. B. Wagener, G. B. Butler, *Journal of Polymer Science: Polymer Letters Edition* **1979**, *17*, 65–77.

[35] K. B. Wagener, S. R. Turner, G. B. Butler, *Journal of Polymer Science: Polymer Letters Edition* **1972**, *10*, 805–816.

[36] Y.-C. Lai, G. B. Butler, *Journal of Macromolecular Science: Part A - Chemistry* **1985**, *22*, 1443–1461.

[37] T. Curtius, *Berichte der deutschen chemischen Gesellschaft* **1893**, *26*, 403–410.

[38] T. Curtius, *Journal für Praktische Chemie* **1894**, *50*, 275–294.

[39] T. Curtius, *Berichte der deutschen chemischen Gesellschaft* **1894**, *27*, 778–781.

[40] K. Shimada, T. Oe, *Analytical Sciences* **1990**, *6*, 461–463.

[41] S. E. Mallakpour, A.-R. Hajipour, H. Raheno, *Journal of Applied Polymer Science* **2002**, *85*, 1141–1146.

[42] S. Mallakpour, H. Nasr-Isfahani, *Indian Journal of Chemistry* **2002**, *41B*, 169–174.

[43] S. Mallakpour, S. Rezazadeh, *Iranian Polymer Journal* **2004**, *13*, 29–38.

[44] S. Mallakpour, Z. Rafiee, *Polymer* **2007**, *48*, 5530–5540.

[45] S. Mallakpour, Z. Rafiee, *Polymer Bulletin* **2006**, *56*, 293–303.

[46] S. Mallakpour, Z. Rafiee, *Journal of Applied Polymer Science* **2007**, *103*, 947–954.

[47] G. Read, N. R. Richardson, *Journal of the Chemical Society Perkin Transactions 1* **1996**, 167–174.

[48] F. Jessica, W. Corentin, D. Sylvestre, L. Christian, L. Andre, *RSC Advances* **2013**, *3*, 24936–24940.

[49] A. Ghorbani-Choghamarani, M. Nikoorazm, G. Azadi, *Chinese Chemical Letters* **2014**, *25*, 451–454.

[50] L. A. Paquette, R. F. Doehner, *The Journal of Organic Chemistry* **1980**, *45*, 5105–5113.

[51] S. Mallakpour, Z. Rafiee, *Synthetic Communications* **2007**, *37*, 1927–1934.

[52] T. J. Gilbertson, T. Ryan, *Synthesis* **1982**, *1982*, 159–160.

[53] G. Zinner, B. Böhlke, *Archiv der Pharmazie* **1966**, *299*, 43–55.

[54] M. J. Bausch, B. David, P. Dobrowolski, C. Guadalupe-Fasano, R. Gostowski, D. Selmarten, V. Prasad, A. Vaughn, L. H. Wang, *The Journal of Organic Chemistry* **1991**, *56*, 5643–5651.

[55] S. Ohashi, G. B. Butler, *The Journal of Organic Chemistry* **1980**, *45*, 3472–3476.

[56] T. Little, J. Meara, F. Ruan, M. Nguyen, M. Qabar, *Synthetic Communications* **2002**, *32*, 1741–1749.

[57] S. Alakurtti, T. Heiska, A. Kiriazis, N. Sacerdoti-Sierra, C. L. Jaffe, J. Yli-Kauhaluoma, *Bioorganic and Medicinal Chemistry* **2010**, *18*, 1573–1582.

[58] M. Shimizu, T. Takahashi, S. Uratsuka, S. Yamada, *Journal of the Chemical Society Chemical Communications* **1990**, 1416–1417.

[59] J. L. Riebsomer, *Chemical Reviews* **1945**, *36*, 157–233.

[60] M. Thiemann, E. Scheibler, K. Wiegand in *Surfactants*, Wiley-VCH Verlag GmbH and Co. KGaA, **2000**.

[61] J. E. Herweh, R. M. Fantazier, *Tetrahedron Letters* **1973**, *14*, 2101–2104.

[62] J. F. W. Keana, A. P. Guzikowski, D. D. Ward, C. Morat, F. L. Van Nice, *The Journal of Organic Chemistry* **1983**, *48*, 2654–2660.

[63] C. Hilger, R. Stadler, *Die Makromolekulare Chemie* **1990**, *191*, 1347–1361.

[64] G. B. Butler, *Industrial and Engineering Chemistry Product Research and Development.* **1980**, *19*, 512–528.

[65] W. Adam, N. Carballeira, *Journal of the American Chemical Society* **1984**, *106*, 2874–2882.

[66] B. Karami, S. Mallakpour, M. Farahi, *Heteroatom Chemistry* **2008**, *19*, 389–393.

[67] M. A. Zolfigol, R. Ghorbani-Vaghei, S. Mallakpour, G. Chehardoli, A. G. Choghamarani, A. H. Yazdi, *Synthesis* **2006**, *2006*, 1631–1634.

[68] M. A. Zolfigol, G. Chehardoli, F. Shirini, S. E. Mallakpour, H. Nasr-Isfahani, *Synthetic Communications* **2001**, *31*, 1965–1970.

[69] A. Khoramabadi-zad, A. Shiri, M. A. Zolfigol, S. Mallakpour, *Synthesis* **2009**, *2009*, 2729–2732.

[70] M. A. Zolfigol, G. Chehardoli, E. Ghaemi, E. Madrakian, R. Zare, T. Azadbakht, K. Niknam, S. Mallakpour, *Monatshefte für Chemie - Chemical Monthly* **2008**, *139*, 261–265.

[71] R. J. Pounder, M. J. Stanford, P. Brooks, S. P. Richards, A. P. Dove, *Chemical Communications* **2008**, 5158–5160.

[72] B. H. Northrop, S. H. Frayne, U. Choudhary, *Polymer Chemistry* **2015**, *6*, 3415–3430.

[73] S. D. Fontaine, R. Reid, L. Robinson, G. W. Ashley, D. V. Santi, *Bioconjugate Chemistry* **2015**, *26*, 145–152.

[74] N. Roy, J. Lehn, *Chemistry–An Asian Journal* **2011**, *6*, 2419–2425.

[75] A. G. Williams, G. B. Butler, *The Journal of Organic Chemistry* **1980**, *45*, 1232–1239.

[76] P. S. Baran, C. A. Guerrero, E. J. Corey, *Organic Letters* **2003**, *5*, 1999–2001.

[77] W. Adam, O. De Lucchi, *Tetrahedron Letters* **1981**, *22*, 929–932.

[78] D. A. Singleton, C. Hang, *Journal of the American Chemical Society* **1999**, *121*, 11885–11893.

[79] A. G. Leach, K. N. Houk, *Chemical Communications* **2002**, 1243–1255.

[80] W. Adam, N. Bottke, B. Engels, O. Krebs, *Journal of the American Chemical Society* **2001**, *123*, 5542–5548.

[81] A. V. Samoshin, C. J. Hawker, J. Read de Alaniz, *ACS Macro Letters* **2014**, *3*, 753–757.

[82] G. B. Butler, *Polymer Science U.S.S.R.* **1981**, *23*, 2587–2622.

[83] I. K. Korobitsyna, A. V. Khalikova, L. L. Rodina, N. P. Shusherina, *Chemistry of Heterocyclic Compounds* **1983**, *19*, 117–136.

[84] C. J. Moody in *Azodicarbonyl Compounds in Heterocyclic Synthesis*, *Vol. 30* (Ed.: A. R. Katritzky), Academic Press, **1982**, pp. 1–45.

[85] M. J. S. Dewar, S. Olivella, J. J. P. Stewart, *Journal of the American Chemical Society* **1986**, *108*, 5771–5779.

[86] F. Carey, R. J. Sundberg, *Advanced Organic Chemistry - Part B: Reactions and Synthesis*, 5th ed., **2007**.

[87] B. Rickborn in *The Retro–Diels–Alder Reaction Part II. Dienophiles with One or More Heteroatom*, John Wiley & Sons, Inc., **2004**.

[88] R. I. Yakhimovich, N. F. Fursaeva, V. E. Pashinnik, *Chemistry of Natural Compounds* **1985**, *21*, 98–103.

[89] M. Fernández-Herrera, J. Sandoval-Ramírez, S. Montiel-Smith, S. Meza-Reyes, *Heterocycles* **2013**, *87*, 571–582.

[90] M. L. Poutsma, P. A. Ibarbia, *Journal of the American Chemical Society* **1971**, *93*, 440–450.

[91] A. P. Henderson, E. Mutlu, A. Leclercq, C. Bleasdale, W. Clegg, R. A. Henderson, B. T. Golding, *Chemical Communications* **2002**, 1956–1957.

[92] J. Zhang, Y. Niu, C. Huang, L. Xiao, Z. Chen, K. Yang, Y. Wang, *Polymer Chemistry* **2012**, *3*, 1390–1393.

[93] X. Chen, M. A. Dam, K. Ono, A. Mal, H. Shen, S. R. Nutt, K. Sheran, F. Wudl, *Science* **2002**, *295*, 1698–1702.

[94] K. Alder, F. Pascher, A. Schmitz, *Berichte der deutschen chemischen Gesellschaft (A and B Series)* **1943**, *76*, 27–53.

[95] K. Mikami, M. Shimizu, *Chemical Reviews* **1992**, *92*, 1021–1050.

[96] M. L. Clarke, M. B. France, *Tetrahedron* **2008**, *64*, 9003–9031.

[97] H. M. R. Hoffmann, *Angewandte Chemie International Edition in English* **1969**, *8*, 556–577.

[98] L. Watson, *PhD Dissertation (Newcastle University)* **2013**, Pericyclic reactions of vinyl–heteroaromatics: multi–component domino and sequential processes.

[99] Y. Hayashi, T. Shibata, K. Narasaka, *Chemistry Letters* **1990**, *19*, 1693–1696.

[100] W. H. Pirkle, J. C. Stickler, *Chemical Communications (London)* **1967**, *1967*, 760–761.

[101] L. J. Watson, R. W. Harrington, W. Clegg, M. J. Hall, *Organic & Biomolecular Chemistry* **2012**, *10*, 6649–6655.

[102] A.-H. Gau, G.-L. Lin, B.-J. Uang, F.-L. Liao, S.-L. Wang, *The Journal of Organic Chemistry* **1999**, *64*, 2194–2201.

[103] A. Pastor, W. Adam, T. Wirth, G. Tóth, *European Journal of Organic Chemistry* **2005**, *2005*, 3075–3084.

[104] M. Squillacote, M. Mooney, J. De Felippis, *Journal of the American Chemical Society* **1990**, *112*, 5364–5365.

[105] M. M. Roubelakis, G. C. Vougioukalakis, Y. S. Angelis, M. Orfanopoulos, *Organic Letters* **2006**, *8*, 39–42.

[106] O. Acevedo, M. E. Squillacote, *The Journal of Organic Chemistry* **2008**, *73*, 912–922.

[107] M. E. Squillacote, C. Garner, L. Oliver, M. Mooney, Y.-L. Lai, *Organic Letters* **2007**, *9*, 5405–5408.

[108] Z. Wang in *Madelung Indole Synthesis*, John Wiley & Sons, Inc., **2010**.

[109] W. Oppolzer, V. Snieckus, *Angewandte Chemie International Edition in English* **1978**, *17*, 476–486.

[110] H. Ban, J. Gavrilyuk, C. F. Barbas, *Journal of the American Chemical Society* **2010**, *132*, 1523–1525.

[111] H. Ban, M. Nagano, J. Gavrilyuk, W. Hakamata, T. Inokuma, C. F. Barbas, *Bioconjugate Chemistry* **2013**, *24*, 520–532.

[112] K. Angermund, R. Bunte, R. Goddard, J. Leitich, O. E. Polansky, M. Zander, *Chemische Berichte* **1988**, *121*, 1647–1650.

[113] R. M. Wilson, N. Chantarasiri, *Journal of the American Chemical Society* **1991**, *113*, 2301–2302.

[114] J. H. Hall, *The Journal of Organic Chemistry* **1983**, *48*, 1708–1712.

[115] J. H. Hall, L. Kaler, R. Herring, *The Journal of Organic Chemistry* **1984**, *49*, 2579–2582.

[116] G. W. Breton, K. R. Hoke, *The Journal of Organic Chemistry* **2013**, *78*, 4697–4707.

[117] G. W. Breton, *Tetrahedron Letters* **2011**, *52*, 733–735.

[118] C. Barbas, H. Ban, J. Gavrilyuk, *US20120289682* **2010**, Tyrosine Bioconjugation through Aqueous Ene–Like Reactions.

[119] W. J. Kinart, C. M. Kinart, R. Oszczeda, Q. T. Tran, *Catalysis Letters* **2005**, *103*, 185–189.

[120] C. A. Seymour, F. D. Greene, *Journal of the American Chemical Society* **1980**, *102*, 6384–6385.

[121] G. W. Breton, J. H. Shugart, C. A. Hughey, S. M. Perala, A. D. Hicks, *Organic Letters* **2001**, *3*, 3185–3187.

[122] C. M. Rasik, M. K. Brown, *Journal of the American Chemical Society* **2013**, *135*, 1673–1676.

[123] J. H. Hall, G. Krishnan, *The Journal of Organic Chemistry* **1984**, *49*, 2498–2500.

[124] H. Wamhoff, K. Wald, *Chemische Berichte* **1977**, *110*, 1699–1715.

[125] A. Christoforou, G. Nicolaou, Y. Elemes, *Tetrahedron Letters* **2006**, *47*, 9211–9213.

[126] R. C. Cookson, I. D. R. Stevens, C. T. Watts, *Chemical Communications (London)* **1966**, 744a.

[127] Q.-Y. Hu, M. Allan, R. Adamo, D. Quinn, H. Zhai, G. Wu, K. Clark, J. Zhou, S. Ortiz, B. Wang, E. Danieli, S. Crotti, M. Tontini, G. Brogioni, F. Berti, *Chemical Science* **2013**, *4*, 3827–3832.

[128] L. H. Dao, D. Mackay, *Canadian Journal of Chemistry* **1979**, *57*, 2727–2733.

[129] W. H. Pirkle, J. C. Stickler, *Journal of the American Chemical Society* **1970**, *92*, 7497–7499.

[130] S. Turner, *PhD Dissertation (University of Florida)* **1971**, Polymerization studies of 4–substituted–1,2,4–triazoline–3,5–diones and synthesis of model compounds related to triple strand polymers.

[131] G. B. Butler, L. J. Guilbault, S. R. Turner, *Journal of Polymer Science Part B: Polymer Letters* **1971**, *9*, 115–124.

[132] L. J. Guilbault, S. R. Turner, G. B. Butler, *Journal of Polymer Science Part B: Polymer Letters* **1972**, *10*, 1–6.

[133] S.-I. Yamamoto, F. Sanda, T. Endo, *Journal of Polymer Science Part A: Polymer Chemistry* **2001**, *39*, 1564–1571.

[134] S. E. Mallakpour, B. Karami-Dezcho, B. Sheikholeslami, *Polymer International* **1998**, *45*, 98–102.

[135] Y.-C. Lai, *PhD. Dissertation (University of Florida)* **1980**, Kinetic, mechanistic and model compound studies of the copolymeriation of bis–4–substituted–1,2,4–triazoline–3,5–diones.

[136] M. Kuhrau, R. Stadler, *Die Makromolekulare Chemie Rapid Communications* **1990**, *11*, 635–644.

[137] M. Kuhrau, R. Stadler, *Die Makromolekulare Chemie* **1992**, *193*, 2861–2874.

[138] M. Kuhrau, R. Stadler, *Polymer International* **1993**, *31*, 71–80.

[139] A. Müller, R. Stadler, *Macromolecular Chemistry and Physics* **1996**, *197*, 1373–1385.

[140] K. B. Wagener, *PhD. Dissertation (University of Florida)* **1979**, Mechanistic, model compound and copolymerization studies of the 4–substituted–1,2,4–triazoline–3,5–dione ring system.

[141] K. B. Wagener, S. R. Turner, G. B. Butler, *The Journal of Organic Chemistry* **1972**, *37*, 1454–1456.

[142] A. G. Williams, G. B. Butler, *Journal of Polymer Science: Polymer Letters Edition* **1980**, *18*, 313–316.

[143] S. E. Mallakpour, J. Asghari, D. Schollmeyer, *Polymer International* **1996**, *41*, 43–52.

[144] S. E. Mallakpour, J. Asghari, *Iranian Polymer Journal* **1996**, *5*, 87–95.

[145] S. E. Mallakpour, H. Kolshorn, D. Schollmeyer, R. Stadler, *Macromolecular Chemistry and Physics* **1997**, *198*, 251–263.

[146] S. E. Mallakpour, F. Mohammdi, H. Kolshorn, *Polymer International* **1997**, *42*, 328–334.

[147] S. E. Mallakpour, A.-R. Hajipour, A.-R. Mahdavian, F. Rafiemanzelat, *Polymer International* **1999**, *48*, 109–116.

[148] S. E. Mallakpour, A.-R. Hajipour, A.-R. Mahdavian, S. Khoee, *Journal of Polymer Science Part A: Polymer Chemistry* **1999**, *37*, 1211–1219.

[149] S. E. Mallakpour, A.-R. Hajipour, A.-R. Mahdavian, *Journal of Applied Polymer Science* **2000**, *78*, 527–536.

[150] S. E. Mallakpour, G. B. Butler, *Journal of Polymer Science Part A: Polymer Chemistry* **1987**, *25*, 2781–2790.

[151] S. E. Mallakpour, G. B. Butler, *Journal of Polymer Science Part A: Polymer Chemistry* **1989**, *27*, 217–235.

[152] E. Baumgartner, *EP0390028A2* **1990**, Graft copolymers and process for producing the same.

[153] B. Ostermayer, *EP0412414A2* **1990**, Thermoplastic moulding compositions.

[154] J. Blackborow, *EP0728766A2* **1996**, Substituted azo–dicarbonylo derivatives.

[155] K. Muehlbach, M. De Araujo, R. Stadler, *EP0338425A1* **1989**, Modifizierte Polyphenylenether.

[156] B. Ostermayer, K. Muehlbach, E. Baumgartner, H. Brandt, H. Feldmann, *EP0370330A2* **1989**, Thermoplastische Formmassen auf Basis von triazolindion-modifizierten Polyphenylenethern.

[157] K.-W. Leong, G. B. Butler, *Journal of Macromolecular Science: Part A - Chemistry* **1980**, *14*, 287–319.

[158] T. C. S. Chen, G. B. Butler, *Journal of Macromolecular Science Part A Pure and Applied Chemistry* **1981**, *16*, 757–768.

[159] R. Stadler, M. M. Jacobi, W. Gronski, *Die Makromolekulare Chemie Rapid Communications* **1983**, *4*, 129–135.

[160] W. Gronski, R. Stadler, M. Maldaner Jacobi, *Macromolecules* **1984**, *17*, 741–748.

[161] L. de Lucca Freitas, M. M. Jacobi, G. Gonçalves, R. Stadler, *Macromolecules* **1998**, *31*, 3379–3382.

[162] C. Hövelmann, B. Gold, A. Bras, J. Allgaier, W. Pyckhout-Hintzen, A. Wischneski, D. Richter, *International Conference on Self-Healing Materials Gent Belgium* **2013**, 688.

[163] R. Stadler, J. Burgert, *Die Makromolekulare Chemie* **1986**, *187*, 1681–1690.

[164] R. Stadler, L. de Lucca Freitas, *Colloid and Polymer Science* **1986**, *264*, 773–778.

[165] L. L. De Lucca Freitas, R. Stadler, *Macromolecules* **1987**, *20*, 2478–2485.

[166] R. Stadler, L. de Lucca Freitas, *Polymer Bulletin* **1986**, *15*, 173–179.

[167] L. de Lucca Freitas, C. Auschra, V. Abetz, R. Stadler, *Colloid and Polymer Science* **1991**, *269*, 566–575.

[168] U. Seidel, R. Stadler, G. G. Fuller, *Macromolecules* **1994**, *27*, 2066–2072.

[169] C. I. D. Bica, W. Burchard, R. Stadler, *Macromolecular Chemistry and Physics* **1996**, *197*, 3407–3426.

[170] C. I. D. Bica, W. Burchard, R. Stadler, *European Polymer Journal* **1997**, *33*, 1759–1766.

[171] M. Müller, U. Seidel, R. Stadler, *Polymer* **1995**, *36*, 3143–3150.

[172] M. Müller, E. W. Fischer, F. Kremer, U. Seidel, R. Stadler, *Colloid and Polymer Science* **1995**, *273*, 38–46.

[173] R. Stadler, L. de Lucca Freitas, *Colloid and Polymer Science* **1988**, *266*, 1102–1109.

[174] U. Seidel, R. Stadler, G. G. Fuller, *Macromolecules* **1995**, *28*, 3739–3740.

[175] R. Stadler, *Macromolecules* **1988**, *21*, 121–126.

[176] M. Mueller, R. Stadler, F. Kremer, G. Williams, *Macromolecules* **1995**, *28*, 6942–6949.

[177] S. Mallakpour, F. Rafiemanzelat, B. Sheikholeslami, *Iranian Polymer Journal* **1997**, *6*, 235–241.

[178] S. E. Mallakpour, A.-R. Hajipour, S. Khoee, B. Sheikholeslami, *Polymer International* **1998**, *47*, 193–197.

[179] L. Freltas, J. Burgert, R. Stadler, *Polymer Bulletin* **1987**, *17*, 431–438.

[180] L. de Lucca Freitas, R. Stadler, *Colloid and Polymer Science* **1988**, *266*, 1095–1101.

[181] R. Wragg, J. Yardley, *GB1456855* **1973**, Method of producing rubber–plastics composites.

[182] G. Pope, P. Smewin, G. Stokes, *DE2342929* **1973**, Carboxylation of unsaturated polymers – by reaction with a carboxy–substd 4–phenyl–1,2,4–triazoline–3,5–dione.

[183] R. Wragg, J. Yardley, *US3899378* **1975**, Method of producing rubber–plastics composites.

[184] C. Hilger, R. Stadler, *Macromolecules* **1990**, *23*, 2095–2097.

[185] C. Hilger, R. Stadler, L. Liane, d. L. Freitas, *Polymer* **1990**, *31*, 818–823.

[186] C. Hilger, R. Stadler, *Die Makromolekulare Chemie* **1991**, *192*, 805–817.

[187] C. Hilger, R. Stadler, *Macromolecules* **1992**, *25*, 6670–6680.

[188] C. Hilger, M. Draeger, R. Stadler, *Macromolecules* **1992**, *25*, 2498–2501.

[189] A. Dardin, R. Stadler, C. Boeffel, H. W. Spiess, *Die Makromolekulare Chemie* **1993**, *194*, 3467–3477.

[190] A. Dardin, C. Boeffel, H. W. Spiess, R. Stadler, E. T. Samulski, *Acta Polymerica* **1995**, *46*, 291–299.

[191] R. Dion, R. Shankar, D. Wilson, M. Malanga, M. Beebe, *WO2008021001A1* **2008**, Process for preparing rubber–reinforced polymer of cyclic monomers.

[192] M. Schirle, J. Beckmann, R. Stadler, *Die Angewandte Makromolekulare Chemie* **1992**, *202*, 261–282.

[193] M. Schirle, I. Hoffmann, T. Pieper, H.-G. Kilian, R. Stadler, *Polymer Bulletin* **1996**, *36*, 95–102.

[194] M. Müller, A. Dardin, U. Seidel, V. Balsamo, B. Iván, H. W. Spiess, R. Stadler, *Macromolecules* **1996**, *29*, 2577–2583.

[195] D. F. Siqueira, J. Reiter, U. Breiner, R. Stadler, M. Stamm, *Langmuir* **1996**, *12*, 972–979.

[196] J. Hellmann, C. Hilger, R. Stadler, *Polymers for Advanced Technologies* **1994**, *5*, 763–774.

[197] A. Dardin, H. W. Spiess, R. Stadler, E. T. Samulski, *Polymer Gels and Networks* **1997**, *5*, 37–54.

[198] R. Stadler, J. Hellman, C. Hilger, *DE4110052A1* **1991**, Modified diene rubbers with thermoplastic elastomer properties.

[199] U. Seidel, J. Hellman, D. Schollmeyer, C. Hilger, R. Stadler, *Supramolecular Science* **1995**, *2*, 45–50.

[200] S. Rout, G. Butler, *Polymer Bulletin* **1980**, *2*, 513–520.

[201] L. L. de Lucca Freitas, M. M. Jacobi, R. Stadler, *Polymer Bulletin* **1984**, *11*, 407–414.

[202] R. Stadler, F. Bühler, W. Gronski, *Die Makromolekulare Chemie* **1986**, *187*, 1301–1312.

[203] R. Stadler, L. L. D. L. Freitas, M. M. Jacobi, *Die Makromolekulare Chemie* **1986**, *187*, 723–729.

[204] R. Stadler, M. Weber, *Polymer* **1986**, *27*, 1254–1260.

[205] C. C. Cheng, C. A. Seymour, M. A. Petti, F. D. Greene, J. F. Blount, *The Journal of Organic Chemistry* **1984**, *49*, 2910–2916.

[206] M. M. Jacobi, R. Stadler, *Die Makromolekulare Chemie Rapid Communications* **1988**, *9*, 709–715.

[207] Y. Zhao, J. Chen, W. Zhu, K. Zhang, *Polymer* **2015**, *74*, 16–20.

[208] S. E. Mallakpour, G. B. Butler, *Journal of Polymer Science Part A: Polymer Chemistry* **1989**, *27*, 125–138.

[209] J. Stock, R. Stadler, *Polymer Bulletin* **1989**, *21*, 319–326.

[210] R. Stadler, M. A. de Araujo, M. Kuhrau, J. Rösch, *Die Makromolekulare Chemie* **1989**, *190*, 1433–1443.

[211] M. Kuhrau, R. Stadler, *Die Makromolekulare Chemie* **1990**, *191*, 1787–1798.

[212] E. Cutts, G. Knight, *US3966530* **1974**, Surface treatment of polymers.

[213] G. Chen, M. Gupta, K. Chan, K. Gleason, *Macromolecular Rapid Communications* **2007**, *28*, 2205–2209.

[214] K. Banert, P. Schumann, *Synlett* **2008**, 535–538.

[215] G. R. Gossweiler, G. B. Hewage, G. Soriano, Q. Wang, G. W. Welshofer, X. Zhao, S. L. Craig, *ACS Macro Letters* **2014**, *3*, 216–219.

[216] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angewandte Chemie International Edition* **2001**, *40*, 2004–2021.

[217] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angewandte Chemie International Edition* **2002**, *114*, 2708–2711.

[218] P. Espeel, F. E. Du Prez, *Macromolecules* **2015**, *48*, 2–14.

[219] J. E. Moses, A. D. Moorhouse, *Chemical Society Reviews* **2007**, *36*, 1249–1262.

[220] C. J. Hawker, V. V. Fokin, M. G. Finn, K. B. Sharpless, *Australian Journal of Chemistry* **2007**, *60*, 381–383.

[221] D. M. Bauer, I. Ahmed, A. Vigovskaya, L. Fruk, *Bioconjugate Chemistry* **2013**, *24*, 1094–1101.

[222] A. Nilo, M. Allan, B. Brogioni, D. Proietti, V. Cattaneo, S. Crotti, S. Sokup, H. Zhai, I. Margarit, F. Berti, Q.-Y. Hu, R. Adamo, *Bioconjugate Chemistry* **2014**, *25*, 2105–2111.

[223] R. Adamo, M. Allan, F. Berti, E. Danieli, Q. Hu, *WO2013009564A1* **2012**, Tyrosine ligation process.

[224] K. Hoogewijs, D. Buyst, J. M. Winne, J. C. Martins, A. Madder, *Chemical Communications* **2013**, *49*, 2927–2929.

[225] M. Shimizu, S. Kamachi, Y. Nishii, S. Yamada, *Analytical Biochemistry* **1991**, *194*, 77–81.

[226] B. Yeung, P. Vouros, G. S. Reddy, *Journal of Chromatography A* **1993**, *645*, 115–123.

[227] B. Yeung, P. Vouros, M.-L. Siu-Caldera, G. S. Reddy, *Biochemical Pharmacology* **1995**, *49*, 1099–1110.

[228] T. Higashi, D. Awada, K. Shimada, *Biological and Pharmaceutical Bulletin* **2001**, *24*, 738–743.

[229] K. Wang, P. P. Davis, T. Crews, L. Gabriel, R. W. Edom, *Analytical Biochemistry* **1996**, *243*, 28–40.

[230] N. Murao, M. Ishigai, N. Sekiguchi, T. Takahashi, Y. Aso, *Analytical Biochemistry* **2005**, *346*, 158–166.

[231] M. A. Tasdelen, *Polymer Chemistry* **2011**, *2*, 2133–2145.

[232] S. Werner, D. P. Curran, *Organic Letters* **2003**, *5*, 3293–3296.

[233] W. Pieken, D. McGee, A. Settle, Y. Zhai, J. Huang, K. Hill, R. Smith, *WO98/47910* **1998**, Method for solution phase synthesis of oligonucleotides.

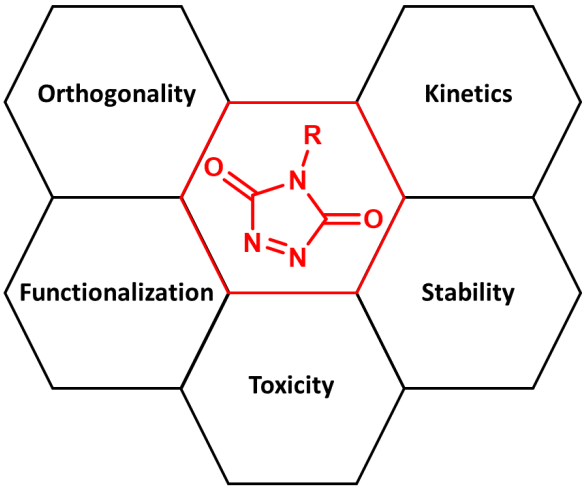
[234] X. Lei, N. Zaarur, M. Y. Sherman, J. A. Porco, *The Journal of Organic Chemistry* **2005**, *70*, 6474–6483.

[235] D. A. Spiegel, F. C. Schroeder, J. R. Duvall, S. L. Schreiber, *Journal of the American Chemical Society* **2006**, *128*, 14766–14767.

[236] L. J. Vanmaele, P. J. De Clercq, M. Vandewalle, *Tetrahedron Letters* **1982**, *23*, 995–998.

[237] I. S. Young, P. S. Baran, *Nature Chemistry* **2009**, *1*, 193–205.

[238] P. S. Baran, C. A. Guerrero, E. J. Corey, *Journal of the American Chemical Society* **2003**, *125*, 5628–5629.



Abstract:

In this chapter an overview of all relevant properties of triazolinediones is given with respect to its general applicability as a synthetic tool. The detailed discussion of relevant features is crucial to understand TAD chemistry and be able to use it in an efficient way. First the orthogonal behaviour of TAD chemistry is discussed, to get a clear view of the reaction conditions that can be applied. Next different strategies are explored to introduce additional functional groups on the TAD molecule. In a next step the kinetics of TAD chemistry are investigated. Finally, both stability and toxicity of the involved molecules are briefly studied.

Chapter III

Investigation of the robustness and general utility of triazolinediones as synthetic tools

III.1 Introduction

In the development of new synthetic methods, the potentially useful reactions are often evaluated on very particular and varying criteria (kinetics, stability...). This makes it obvious that the required or desirable reaction characteristics can widely vary in different fields and strongly depend on the application. Most synthetic methodologies in the scientific literature nowadays focusses on the reaction yield. Although this is a simple quantitative measure, this can be influenced by many factors (including the skill set of the involved chemist).[1] For this reason, the chemical community prefers reactions that are robust and perform well under various conditions. However, a major issue in the development of new synthetic methodologies is often the lack of information concerning the general chemical and other properties that can influence the success of a reaction (especially in non-optimized environment).[2] Important considerations in this respect are orthogonality, reaction kinetics and compound stability and toxicity of starting materials, intermediates and obtained adducts. Collins and Glorius recently discussed this matter extensively and urged the scientific world to use a simple and fast assessment of 'new' chemical reactions.[1,2]

Like with most breakthrough applications, innovative ideas are often based on old knowledge that is reintroduced into another field and/or used for other purposes. The development of this doctoral work was no exception to this. The first use of 1,2,4-triazoline-3,5-dione (TAD) as an efficient reactant in organic synthesis originates back to literature older than 50 years.[3] Shortly after, TAD chemistry introduced itself to polymer science.[4] Since then, a number of different applications were extensively studied (as discussed in chapter II). Although some attempts had been made to map the synthetic robustness and general utility of these remarkable molecules[5–7], most of the literature focusses on the reactivity of TAD while valuable, basic information such as orthogonality, stability, kinetics... has not been investigated systematically.

This chapter will try to give an overview of the (for this work) relevant properties of triazolinediones. In this way, a solid understanding of the TAD molecule - in its broadest sense - is pursued.

First the orthogonality of both the Diels-Alder (DA) and the reversible Alder-*ene* (AE) reaction will be investigated. This is crucial to determine reaction conditions in which a given reaction involving TAD can proceed without any problem.

In the next part, the introduction of functionalities on the TAD molecule itself is described. The number of commercially available TAD molecules is limited. In fact, most examples in literature stick to this limited range of TAD molecules. Therefore, a major part of this work consists to find out easy (and upscalable) synthesis routes for (new) functional TAD molecules.

As TAD can undergo a wide span of reactions in complex reaction media, it is primordial to determine first its reactions kinetics, allowing one to choose an appropriate reaction partner as a function of the desired reaction rate and/or end product. Chapter II described the reactivity of TAD, while here an overview will be given of the kinetics of TAD in combination with its most relevant reaction partners (for DA and AE reactions) using both literature data and new investigations.

The stability of TAD and its adducts has also been addressed in this work.

Finally, the toxicity of TADs will be shortly discussed, in view of the potential use of

TAD chemistry in biomedical applications.

III.2 Orthogonality study of TAD reactions

Maybe the most crucial part of information for the general application of a synthetic methodology, is its tolerance towards functional groups. When chemical reactions are performed in a wide application range, reaction media will differ to a certain extent. Other molecules (solvent, additives, catalyst...) can interfere in the reaction as the application can be quite different from earlier found optimized conditions.[7] In methodologically driven research, non-reactive side groups are introduced to the starting materials showing its tolerance to specific chemical functionalities. Despite the fact that these procedures are well established, they still show some limitations. The main problem lies in the fact that non-reactive functional groups can possibly influence the reaction centre, making it difficult to predict if the obtained knowledge can be extrapolated to more complex reaction media.

To face this difficulty, Collins and Glorius considered in 2013 a general robustness screening for chemical reactions.[2] Their described method intentionally decouples the functional group from the reactive centre and treats it as an additive (see Figure III.1). This approach will provide correct data concerning the functional group and also shows the limitations of the chemical transformations.

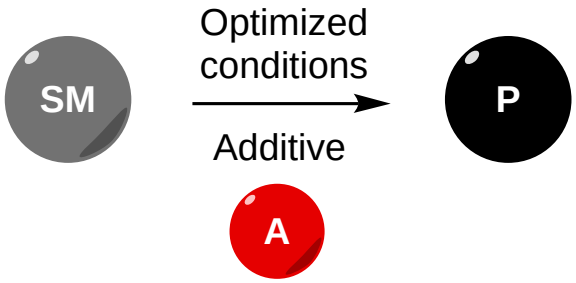


Figure III.1: Chemical robustness test based on the addition of a functional group as additive (A) – SM = starting material, P = product (drawing adapted from ref.[2])

For this work the focus will be on two reactions: the (irreversible) Diels-Alder reaction and the (reversible) Alder-*ene* reaction (between TAD and indole). Both reactions have been introduced in chapter II (see II.3.1 and II.3.2) and will be discussed in detail in chapter IV and chapter VI respectively.

III.2.1 Diels-Alder reaction

To demonstrate the tolerance and robustness of the TAD-diene Diels-Alder reaction (DA), a reaction between 4-butyl-1,2,4-triazoline-3,5-dione (BuTAD, **31**) and a commercially available diene, 2,4-hexadien-1-ol (HDEO, **32**) in DMSO-d_6 was studied (see Figure III.2).

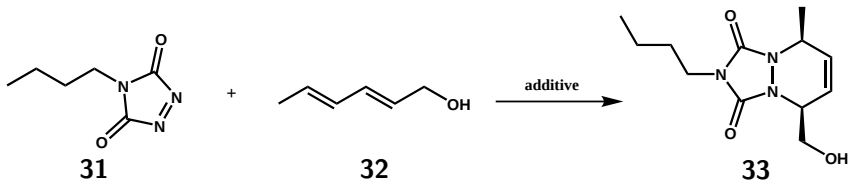


Figure III.2: Standard reaction between BuTAD (**31**) and HDEO (**32**) in the presence of an additive, to check the robustness of the irreversible TAD DA reaction.

As this diene already contains an alcohol functionality and it is known form literature that TAD can react with alcohols[8], a preliminary test with **31** and **32** was first performed to verify if any interference could occur. The characteristic red colour of **31** disappeared instantaneously upon mixing the reagents and via ^{1}H NMR it was determined that a clean conversion had occurred (see Figure III.3). Due to the clean NMR spectrum (no column chromatography) and the limited synthetic effort, this reaction was chosen as model for the DA reaction.

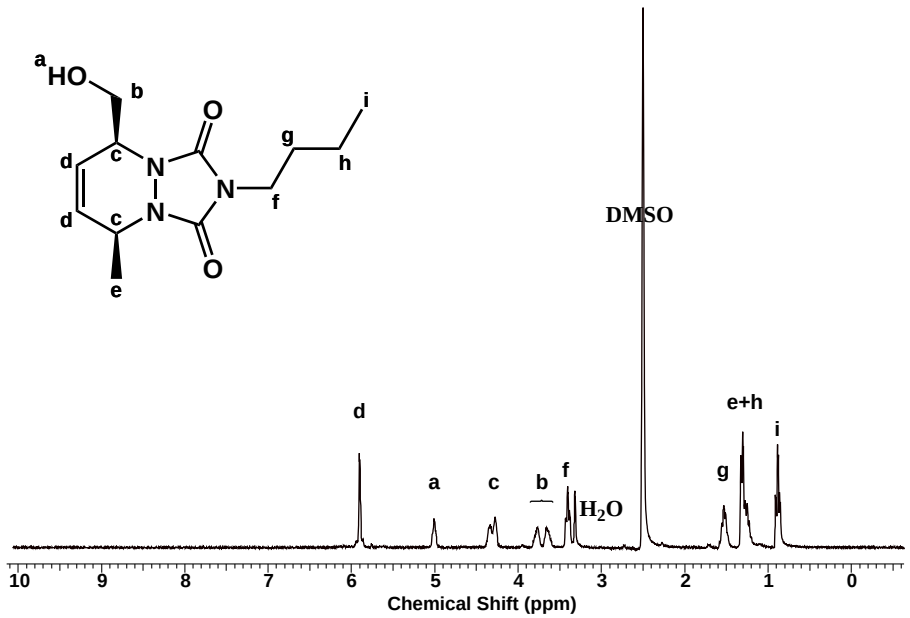
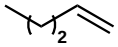
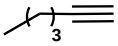
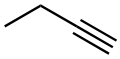
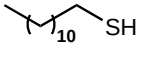
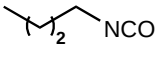
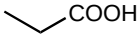
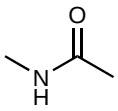
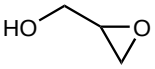
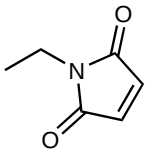
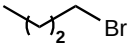
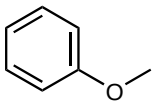
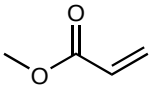
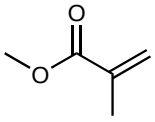
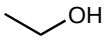
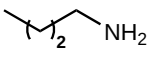


Figure III.3: ^{1}H-NMR spectrum of the Diels-Alder adduct (**33**) of BuTAD (**31**) and 2,4-hexadien-1-ol (**32**) (300 MHz - DMSO d_6 + residual H_2O).

Table III.1: Orthogonality study for the reaction between BuTAD (**31**) and HDEO (**32**).

Entry	Additive	Yield of 33 (%)	Additive remaining (%)	Remaining 32 (%)
1		100	100	0
2		100	100	0
3		100	100	0
4		100	100	0
5		100	100	0
6		100	100	0
7		100	100	0
8		100	100	0
9		100	100	0
10		100	100	0
11		100	100	0
12		100	100	0
13		100	100	0
14		100	100	0
15		100	100	0
16		95	95	5
17		95	95	5
18		0	0	100

The standard reaction was performed in the presence of one molar equivalent of a given 'additive' (= organic molecule containing functional group). For this a 1:1 molar mixture of HDEO (**32**) and additive in DMSO-d_6 is prepared and to this, a solution of one equivalent of BuTAD (**31**) is added. The amount of remaining additive, after reaction, is given in Table III.1 (the yield was determined by ^{1}H-NMR analysis). Colour coding is added to help the assessment of the provided data (Green '$\vee$' (tolerant), yellow '-' (some interference) and red '$\times$' (not compatible)).

As can be seen in Table III.1, the functional group tolerance is high (entries 1-15). It is known that some additives can react with TAD[7,8] however in the presence of a diene TADs favour the *ultrafast* DA reaction. This can be defined as a form of '*kinetic orthogonality*', as without a diene TAD molecules would react completely with some of the additives.

There are some exceptions to this orthogonality as can be seen in entries 16-18. Firstly, the presence of amines in the reaction mixture disturbs the standard reaction slightly (as can be expected from chapter II) Via NMR it was determined that around 5% side reactions occur. The exact mechanism of this side reaction has been discussed already in section II.3.5.

The last entry shows that the standard reaction is not compatible with furan. Furan, also a diene, can react with TAD in a range of different reactions (Diels-Alder[7], Electrophilic Aromatic Substitution[9]...). And thus not surprisingly, when the reaction between BuTAD (**31**) and furan was attempted, a complex mixture of products was obtained.

III.2.2 TAD-Indole Reaction

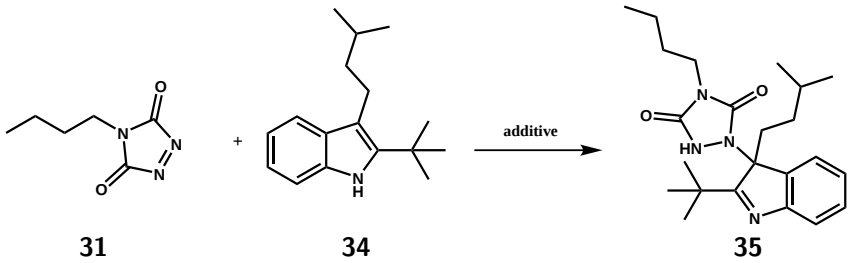
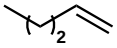
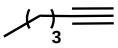
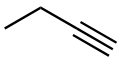
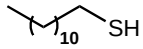
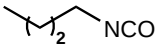
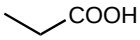
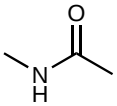
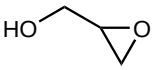
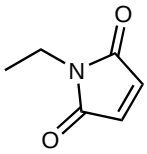
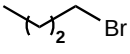
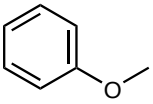
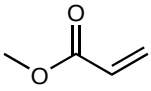
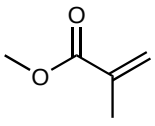
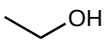
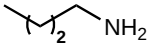


Figure III.4: Reaction between BuTAD (**31**) and 2-*tert*-butyl-3-isopentyl-1*H*-indole (**34**) in the presence of various additives.

Parallel to the irreversible DA reaction, also the (reversible) AE reaction (see chapter

Table III.2: Orthogonality study for the reaction of BuTAD (**31**) and 2-*t*-Bu-3-isopentyl-1*H*-indole (**34**).

Entry	Additive	Yield of 35 (%)		Additive remaining (%)		Remaining 34 (%)
1		100	✓	100	✓	0
2		100	✓	100	✓	0
3		100	✓	100	✓	0
4		100	✓	100	✓	0
5		100	✓	100	✓	0
6		100	✓	100	✓	0
7		100	✓	100	✓	0
8		100	✓	100	✓	0
9		100	✓	100	✓	0
10		100	✓	100	✓	0
11	H_2O	100	✓	100	✓	0
12		100	✓	100	✓	0
13		100	✓	100	✓	0
14		100	✓	100	✓	0
15		100	✓	100	✓	0
16	NEt_3	0	✗	0	✗	100
17		0	✗	0	✗	100
18		0	✗	0	✗	100

VI & VII) was followed to check functional group tolerance. Here, the reaction between BuTAD (**31**) and 2-*tert*-butyl-3-isopentyl-1*H*-indole (**34** - an 1*H*-indole variant that does not contain any functional groups) was investigated in detail (see Figure III.4). The red colour of BuTAD disappeared in more or less one minute upon mixing the reagents at room temperature. Via ^{1}H NMR the conversion was determined.

Table III.2 gives an overview of the functional group tolerance for the standard reversible AE reaction. Again entries 1 to 15 showed excellent orthogonality and the same exceptions as in III.2.1 were found (entries 16-18). In contrast to the DA reaction (where only furan proved unsuccessful), here both amines and furan severely alter the outcome, defining these components as undesired in the reaction mixture.

This orthogonality study shows promising results for both reactions. A whole range of functional groups can be present on the substrate or in the reaction media without disturbing the reactions, making it possible to introduce both synthetic reactions in polymer materials.

III.3 Introduction of functionalities on TAD

Due to the limited commercial availability of TAD components (only 4-phenyl-1,2,4-triazoline-3,5-dione (PhTAD, **1**) is readily available at this time), there is a huge need to synthesize them in house. Crosslinkers and non-functional TAD molecules can be easily prepared by following literature procedures starting from isocyanates (see section II.2).[10] The previous section (III.2) showed that TAD is very tolerant towards functional groups, hence the introduction of them onto TAD molecules seemed plausible. However, when starting from isocyanates, the synthetic routes are rather limited, luckily there are other ways to prepare TAD molecules - as discussed in chapter II. Prior to this doctoral work, research groups started synthesizing TAD components containing additional functional handles. Unfortunately the known examples remain small scale and specific to a certain project.[11–14] Therefore, general and upscalable synthesis routes were envisaged for the most relevant functional groups.

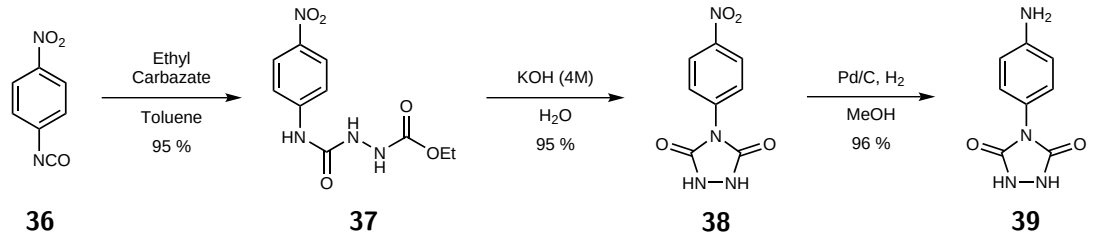
For this doctoral work the focus lies on the preparation of TAD chemicals containing amines, alcohols or groups that serve as polymer initiator (for Cu-mediated polymeriz-

ation) or mediating agent (for RAFT polymerization). To avoid reaction between the introduced functional group and the TAD moiety, the synthesis is stopped at the urazole level. In this way functionalization making use of the functional group can proceed with a significant lower chance of side reactions to occur.

III.3.1 Synthesis of amino-functionalized urazoles

Based on the orthogonality described in the previous section, it may seem counterintuitive to go for an amino-moeity as a functional group. To circumvent this issue, not the TAD molecule itself but the urazole precursor will be synthesised and an amine functionalization will be performed at this stage (i.e. an amide bond formation). Once the wanted amide-type connection has been made, the respective TAD moiety can be generated via oxidation (see chapter II.3).

Taking into account the commercial availability of the starting products, a first attempt was made to produce aniline containing urazoles (see Figure III.5). For this 4-nitrophenyl isocyanate (**36**) was treated with one equivalent of ethyl carbazate in toluene to produce the corresponding semi-carbazide (**37**) in high yield (95%). The next step, the ring closure was performed in basic media leading to 4-nitrophenyl urazole (**38**, 95%). In a last step, the nitro group was reduced to an amine using a catalytic amount of palladium (5% on activated carbon) and a hydrogen atmosphere (96%). This rather straightforward method leads to the desired aniline containing urazole (**39**) in an upscalable way (up to 25 grams in the lab).



36 **37** **38** **39**

Figure III.5: Reaction scheme for a urazole containing an aromatic amino-group from 4-nitrophenyl isocyanate (**36**).

Unfortunately, only a limited amount of reactions was possible with this aniline derivative (see later), so it became clear that a general method for the introduction of an aliphatic amino-function was necessary. In a first attempt, 6-aminocaproic acid (**40** - a derivative from the amino acid lysine) was chosen as starting product. Due to possible interfer-

ence of the amine functionality in the process, it seemed appropriate to first protect this functional group. A broad range of protecting groups are used for amines. In this case the use of benzyl carbamate (Cbz) proved to be a success giving the Cbz-protected 6-aminocaproic acid (**41**) in good yields (95%).[15] Then the carboxylic acid underwent a *Curtius rearrangement* in which the azide of diphenyl phosphoryl azide (DPPA) attacked the carboxylic acid to form an acyl azide that subsequently rearranged to the corresponding isocyanate by means of heating. The *in situ* formed isocyanate is then reacted with ethyl carbazate, giving the desired Cbz-protected semi-carbazide (**42** - see Figure III.6). Different conditions were attempted to perform the urazole ring closure. The best results were obtained using four equivalents of potassium carbonate (K_2CO_3).[12] In order to obtain a pure compound (**43**) flash chromatography was performed (ethyl acetate : heptane - 40% to 100% EtOAc). It should be emphasized that column chromatography was not necessary for the synthesis of many other TAD compounds, however it proved to be crucial. As a final step the protecting group was removed using literature procedures,[12] albeit in rather low yield (**44**, <50% - (presumably) due to catalyst poisoning). Later, this last step was optimized by adding two equivalents of acid (HCl, thionyl chloride...) to the starting mixture already containing methanol, palladium and urazole. By adding acid to the solution the formed amine is protonated, making it impossible to poison the catalyst.

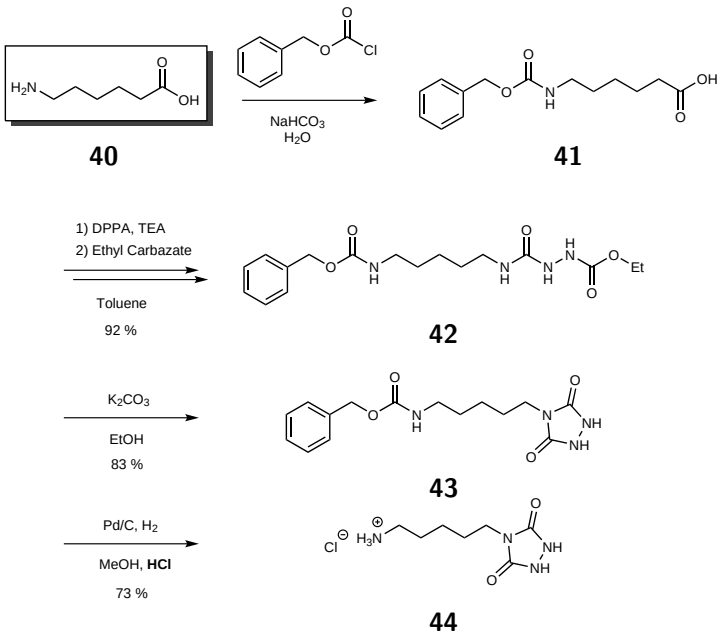


Figure III.6: Synthesis of an aliphatic amine containing a urazole moiety starting from 6-aminocaproic acid (**40**).

While this seemed an interesting approach, some problems surfaced during upscaling. Especially the removal of water (solvent) after the first step, proved to be tedious when working on larger scale. A solution was found in literature[16] offering an alternative approach starting from the economically interesting hexamethylene diisocyanate (HDI, **45**) as starting product. A one-pot reaction with a mixture of HDI, benzyl alcohol and ethyl carbazate in toluene gave three different products (as can be seen in Figure III.7). The desired product (**48**, 19%) was obtained together with two side products, the bis-semicarbazide (**46**, 33% - that can be used for the synthesis of an aliphatic crosslinker), and the bis-Cbz-protected bis-amine (**47**, 30% - can also be used for the synthesis of an aliphatic crosslinker after deprotection) and then isolated via column chromatography (eluent = ethyl acetate:heptane 2:3 to 100 % ethyl acetate). Although the yield is not that high, this is an easy to scale method that produces besides the desired compound, two other products are of high value in this project. Optimization of this reaction is still under investigation in our group. With the desired Cbz-protected semi-carbazide (**48**) prepared, the same steps were performed as in the original procedure (see Figure III.6) leading to a urazole containing an aliphatic amine (**49**). It has to be noted that due to the different starting molecules, one extra carbon is introduced between the amine and the urazole moiety.

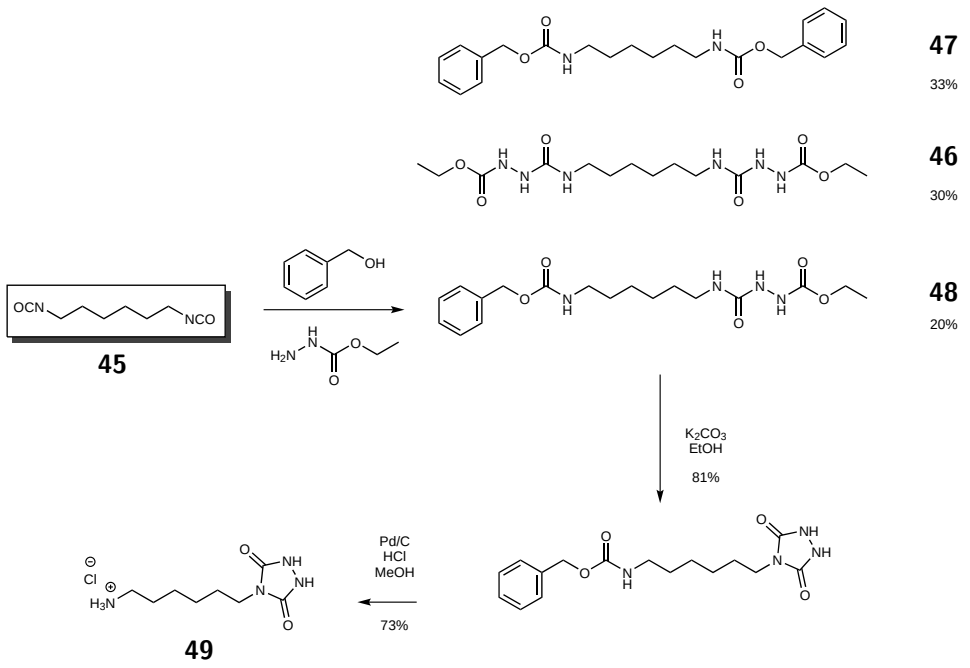


Figure III.7: Alternative synthesis route of a urazole containing an aliphatic amino-group starting from hexamethylene diisocyanate (**45**).

III.3.2 Synthesis of hydroxyl-functionalized urazole

Besides amines as useful functional groups, also hydroxyl containing urazoles are of particular interest. Stadler *et al.* published in 1987 a synthesis route for 4-hydroxyl-substituted PhTAD.[17] However, this four-step synthesis was difficult to reproduce and to scale up. Taking into account the data coming from the amino-functional TAD derivatives, the preference goes to an aliphatic hydroxyl instead of its phenolic counterpart. Again the use of protecting groups was necessary to provide an efficient and upscalable route. The protecting group of choice (due to the ease of deprotection) this time is a benzyl ether. Figure III.8 shows a procedure starting from the commercially available 4-benzyloxybutyric acid (**50**). The synthesis pathway is similar to the route of the amino-functionalized urazole (*Curtius rearrangement*, addition of ethyl carbazate and basic ring closure, respectively) albeit with one distinct difference. The deprotection is no longer possible with Pd/C as reducing agent for unknown reasons. Luckily an alternative reducing agent (palladium hydroxide – $Pd(OH)_2$) proved to be a valid alternative, leading to pure end product (**51**) in a decent yield (82%) after precipitation.

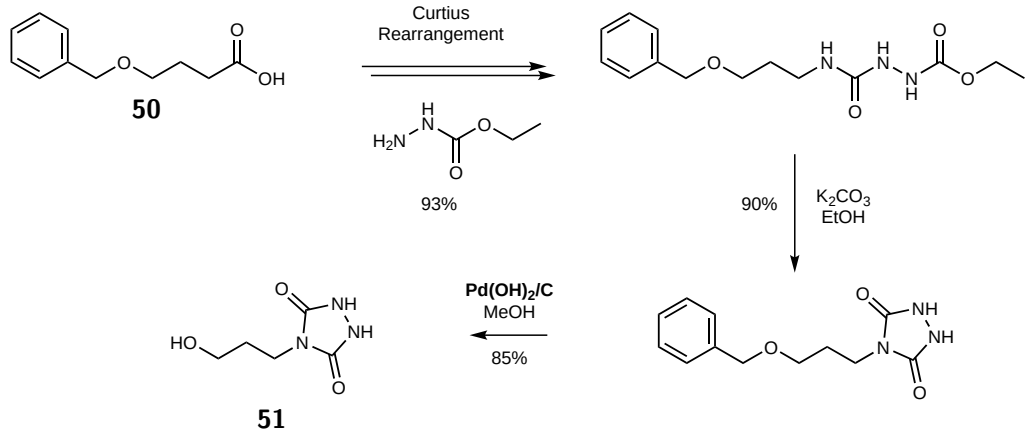


Figure III.8: Proposed reaction scheme for the synthesis of a urazole containing an aliphatic hydroxyl-group starting from 4-benzyloxybutyric acid (**50**).

The only drawback connected to this described synthesis route is the cost of the starting product (4-benzyloxybutyric acid, **50**). Therefore an additional source of starting products was investigated. In this respect, the choice for ϵ-caprolactone (**52**) was easily made. This lacton (bulk chemical, seven membered ring) is used as a monomer in the synthesis of polycaprolacton. When ϵ-caprolactone is treated with benzyl bromide (**53**) this leads to the formation of a hetero-bifunctional molecule (**54** - see Figure III.9)

containing a benzyl ether and a carboxylic acid.[18] The continuation of the process to obtain the alcohol containing urazole (**55**) is the same as the example described above (Figure III.8). Due to the change in starting compound an extra spacer is introduced between the functional group and the urazole. This flexibility would be convenient when the molecule will be used in a polymer context.

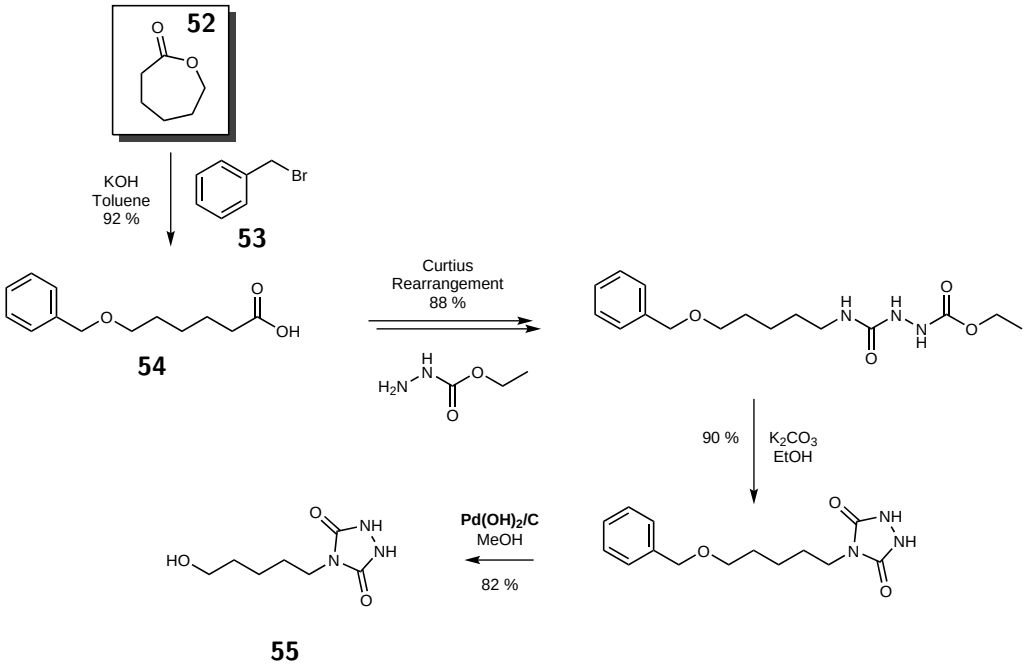


Figure III.9: Reaction scheme for the synthesis of a urazole containing an aliphatic hydroxyl-group starting from ϵ-caprolactone.

III.3.3 Synthesis of polymer initiator and chain transfer agent containing a urazole

Introducing TAD molecules in polymer science is not a trivial task. Due to the lack of functional handles in easily available TAD reagents, the main examples have been focused on functionalization of polymers with PhTAD[19] or crosslinking (see chapter II.4).[20] An important remark is that the focal point of the research concerning TAD molecules in polymers precedes the introduction of controlled radical polymerizations (CRP). Since in the Polymer Chemistry Research (PCR) group the focus nowadays lies on *Cu(0) mediated* and *Reversible Addition-Fragmentation chain Transfer* (RAFT) polymerization, an initiator and chain transfer agent for both polymerization techniques were developed in this work. This allows the easy introduction of polymer chains as a 'functional moiety' on TAD.

III.3.3.1 Synthesis of an initiator for *Cu(0) mediated* polymerization

Copper(0) mediated polymerization mainly makes use of alkyl halides R-X (X = Cl, Br) as initiator. Such alkyl halides may also contain various functional groups. The most important advantages of using functional initiators in the synthesis of Cu(0) mediated polymerization are the direct functionalization and the fact that it avoids a post-polymerization modification.[21] This opens opportunities to create an initiator containing urazole moieties (that can be oxidized to TAD after polymerization).

The simple urazoyl-aniline (**39**) proved useful for the synthesis of an initiator. It is dissolved in dry pyridine and cooled down to 0 °C. To this solution α-bromoisobutyryl bromide is added dropwise. The mixture is brought to room temperature, followed by stirring overnight and column purification (eluent = (ethyl acetate:methanol:acetic acid; 95:5:1)(petroleum ether) (2:1)) to obtain the desired urazole initiator (**56** - see Figure III.10 - for detailed description of the compound see III.8.3.9).

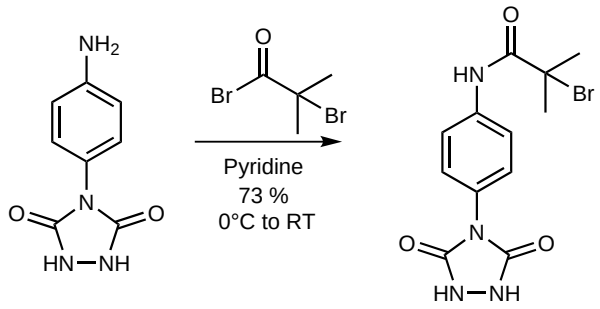


Figure III.10: Synthesis of an initiator for Cu(0) mediated polymerization.

III.3.3.2 Synthesis of a chain transfer agent for RAFT polymerization

The RAFT process can be generally described as a controlled radical polymerization technique of a vinyl monomer in the presence of a suitable chain transfer agent (RAFT agent or CTA). Commonly used RAFT agents include thiocarbonylthio compounds such as dithioesters, dithiocarbamates, trithiocarbonates and xanthates, which mediate the polymerization via a reversible chain-transfer process.[22] For this work, a urazole containing a trithiocarbonate function was selected as targeted compound. Moreover, these CTAs show high transfer constants, possess high hydrolytical stability and are compatible with styrenes, acrylates and acrylamides.

The synthesis of this CTA initially proved to be quite problematic. Many strategies to form a trithiocarbonate in a urazole substrate were attempted yet none were successful. Therefore, our focus shifted to the coupling of an already synthesised trithiocarbonate to a functional urazole. In this work, 2-([butylsulfanyl-carbonothioyl]sulfanyl)-propanoic acid (BuPAT, **57**) was used as the RAFT agent, for a number of reasons. First of all, the large-scale synthesis of BuPAT is high yielding.[23] Secondly, BuPAT has a carboxylic acid functionality that can be used as a synthetic handle to couple the urazole. However, due to acidic protons of the urazole moiety interference occurs when coupling is attempted with hydroxyl- and amino-urazole derivatives. Thus, the activated ester of BuPAT (**58**) was first prepared.[24] This could be very efficiently coupled to the aliphatic amine urazole (**49**) to yield the pure urazole CTA (**59** - see Figure III.11 - for detailed description of the compound see III.8.3.10)) after column chromatography (eluent = ethyl acetate:methanol; 95:5). This RAFT CTA proved to be effective (controlled polymerization) as this is currently employed in an ongoing PhD study within our own research group (Stef Vandewalle).

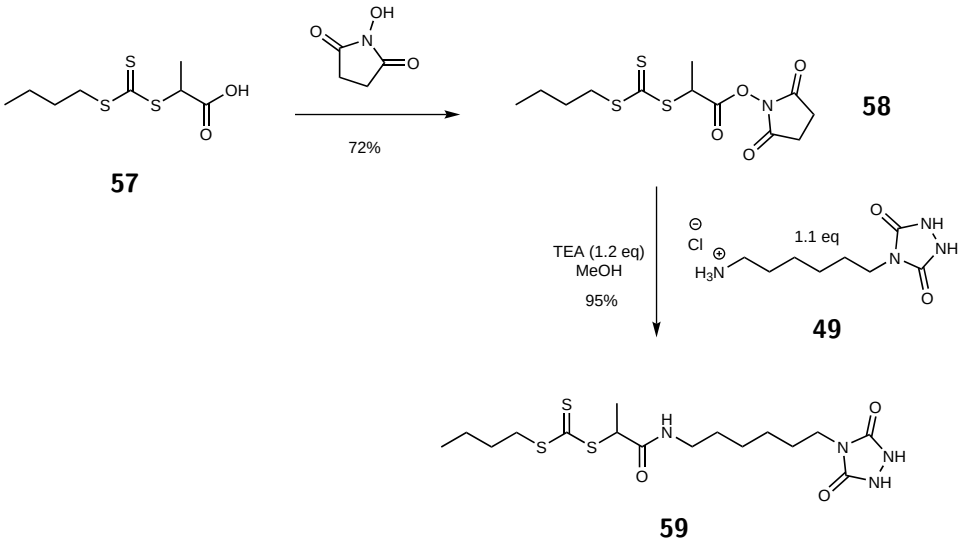


Figure III.11: Synthesis of a urazole CTA for RAFT polymerization.

III.4 TAD reaction kinetics

In order to get a full understanding of the kinetic behaviour of TAD molecules, the electronic structure of these molecules has to be considered in detail. Similar to singlet carbenes and singlet oxygen, TAD contains two orthogonal *p-type* orbitals of very similar energy on the same atom(s), only one of which is occupied. This dual character is the basis of the remarkably reactivity. Houk *et al.* showed that TAD indeed possesses a high-energy π^* HOMO (gives a possible nucleophilic character – see Figure III.12a) and a low-lying π^* LUMO (the main source of the electrophilicity of TAD – see Figure III.12b) that is geometrically orthogonal to the HOMO.[25] The energy gap between the HOMO and LUMO is rather small, as both are anti-bonding combinations (π^*) of N-atom p-orbitals, which is reflected by the intense colour of TAD molecules.

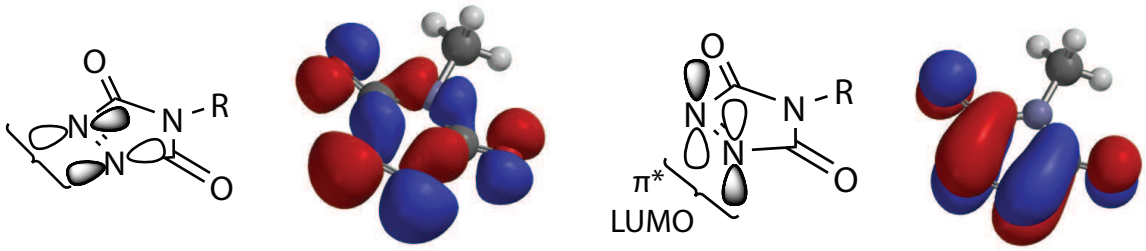
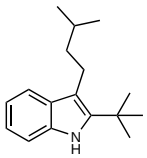
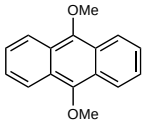
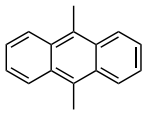
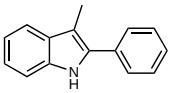
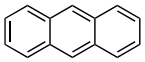
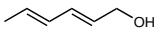
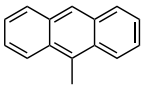
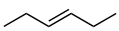
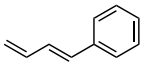
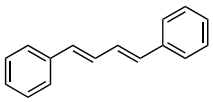
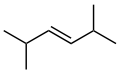
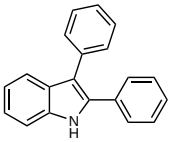
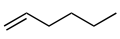


Figure III.12: a) High-energy occupied π^*-type molecular orbital (HOMO) and b) an unoccupied π^*-type MO (LUMO) of very similar energy. These anti-bonding MO's are orthogonal and mainly localised on the azo-N-atoms.

As can be seen in Table III.3, a great deal of data on the DA and AE reaction between PhTAD and different dienes and alkenes has already been reported.[26–28] The observed trend can be explained by a combination of two factors, being the geometry of the transition state and the higher HOMO due to conjugation of the π-bond and respective C-H σ-bond (AE reaction - see Figure III.13) or C-C π-bond (DA-reaction). In general, it can be seen that dienes have a more favourable kinetic behaviour than *enes*. However it was already reported by Butler that an increase in alkylation of the involved *ene* leads to faster reactions due to the presence of electron donating groups (EDG) on the double bond (leading to the higher HOMO).[29] This effect can be so strong that in the case of a tetra-substituted double bond (for example: 2,3-dimethyl-2-butene) the reactions kinetics are in the same range as some conjugated double bonds.[27]

Table III.3: Rate constants (k_2) at room temperature for a variety of substrates in their reaction with PhTAD (a = toluene, b = dichloromethane, c = acetone, d = benzene and e = chloroform).[26–28,30]

Substrate	k_2 ($M^{-1}s^{-1}$)	Substrate	k_2 ($M^{-1}s^{-1}$)
	160 000[a]		0.434[b]
	13 750[a]		0.38[c]
	12 180[a]		0.378[c]
	333[b]		0.33[a]
	242[c]		0.194[b]
	180[d]		0.18[b]
	84[a]		0.15[b]
	63.1[d]		0.123[b]
	51.9[e]		0.05[b]
	46[d]		0.038[b]
	6.92[d]		0.012[c]
	6.5[b]		0.012[b]
	5.13[b]		0.01[b]
	1.92[b]		0.00395[a]
	0.89[b]		0.000733[b]
	0.527[b]		

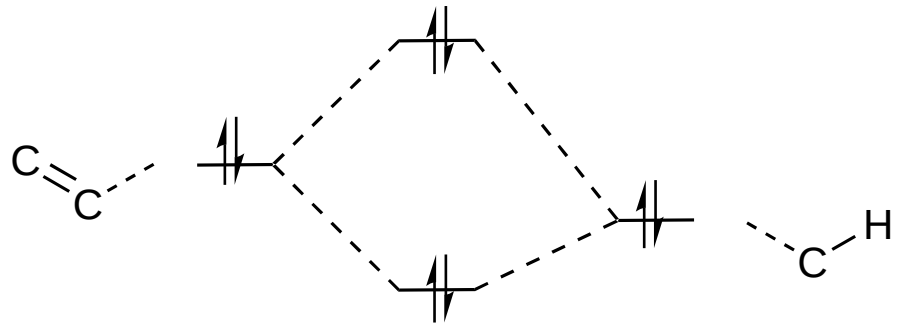


Figure III.13: Conjugation of double bond with a C-H σ-bond (AE reaction) leading to higher HOMO.

The obtained knowledge gives, in combination with an extensive literature search, an excellent opportunity to compare a wide range of different substrates in their kinetic behaviour with TAD. Table III.3 shows that cyclopentadiene is the most reactive substrate. This can be expected because of the locked *s-cis* conformation of the diene. For a long time it was not possible, due to the very rapid reaction (instantaneously even at -78 °C), to determine the involved rate constant. It was not until very recently, when Kisilev *et al.* used stopped-flow UV measurements (see III.8.2 for experimental details), a value of 160 000 $M^{-1}s^{-1}$ was established.[30] This huge rate constant was in agreement with previous qualitative analysis that showed when two dienes were present, cyclopentadiene would always be the preferred partner.[3]

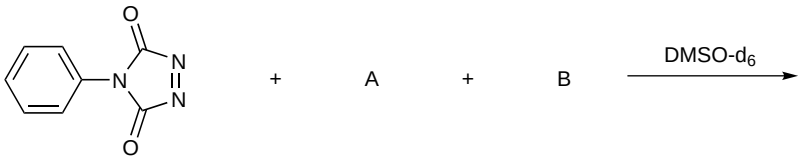
In addition to the existing literature, some additional experiments had to be conducted in order to give a complete overview of the used substrates in this work. For this two sets of experiments were conducted.

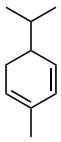
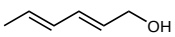
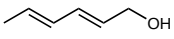
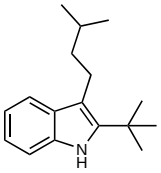
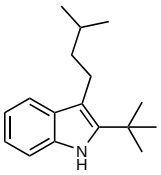
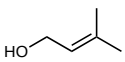
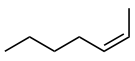
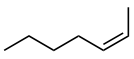
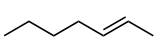
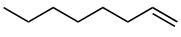
First, Table III.3 was completed with rate constants of the reaction between PhTAD and (for this work) relevant molecules. This was done by means of the *initial rate method*[31] for three 1*H*-indoles – specific *enes* that show reversible behaviour with TAD moieties (see chapter VI and VII) and one diene (HDEO). HDEO is a cheap, commercially available diene with a functional handle, making it a suitable component to be introduced in a variety of polymer materials, as can be seen in chapter IV. The main advantage of compiling a kinetic hierarchy, as in Table III.3, is the ease of predicting the main reaction product in more complex reaction media. Moreover, it is shown that TAD reactions differ greatly in reaction rate purely based on the chosen substrate (differences of 8 orders of magnitude in terms of rate constant!). This opens opportunities to choose a complementary partner,

based on the kinetics, as a function of the desired properties.

Secondly, a more qualitative test (a *head-to-head* analysis) was performed. In this experiment, two substrates were dissolved in DMSO-d_6 and mixed in an equimolar ratio. To this one equivalent of PhTAD was added dropwise. After the characteristic red colour of PhTAD had disappeared, a ^{1}H NMR measurement was performed. Out of these measurements the relative ratio of the two formed adducts was calculated (see Table III.4).

Table III.4: *Head-to-head* analysis: Reaction between PhTAD and two substrates in equimolar ratio. Relative rates of the respective end products are given (%) as determined via ^{1}H NMR.



Substrate A	**Product of PhTAD & A (%)**	**Substrate B**	**Product of PhTAD & B (%)**
	61		39
	100		0
	100		0
	69		31
	60		40
	58		42
	88		12

When developing a 'new' platform it makes sense to compare this to existing systems, to benchmark the new technology. This comes down to comparing TAD reactions to existing *click* chemistries as a function of their kinetic behaviour. Table III.5 gives an overview of the fastest reported rate constants for some of the most known *click* reactions such as (radical) thiol-ene, copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) and others.

Fox *et al.* reported in 2008 the fastest *click* reaction known until now, with rate constants going up to $2000\,M^{-1}\,s^{-1}$. This tetrazine ligation is based on an inverse-electron-demand DA reaction between *s*-tetrazine and *trans*-cyclooctene. Interestingly TAD chemistry can provide much larger rate constants (up to $160\,000\,M^{-1}s^{-1}$) if the substrate is chosen well, making the TAD based *click* chemistry one of the fastest known. This is of course an excellent starting point to investigate the *click* character of this chemistry in full detail (see chapter IV).

Table III.5: Relative comparison of the fastest reported rate constants of known *click* reactions.

FAST		**MODERATE**		**SLOW**	
Tetrazine ligation[32]	**Dithioester-Cp**	**CuAAC**[33]	**Strained promoted AAC**[34]	**Radical thiol-*ene***[35]	**Furan-maleimide**[36]
2000	>1	1.0	0.96	10^{-3} – 10^{-6}	1.93 10^{-5}

III.5 Stability of TAD compounds

When using a very reactive compound, such as TAD, it is crucial to have effective storage conditions. This way, the compound can be prepared well before the actual reaction is required. In order to come to adequate conditions for storage, the stability of TAD has to be looked at in detail. Based on literature, it becomes clear that TAD moieties should avoid higher temperature, water and strong light sources.[7,37] In what follows a more detailed overview will be given why these circumstances must be avoided.

III.5.1 Thermal Stability

As already mentioned in II.3.5, TAD can decompose at elevated temperature to form a dimer. Wamhoff *et al.* described in 1977 the decomposition of PhTAD (**1**). It was shown that at a temperature of 150 °C, nitrogen gas is released leading to a diradical. This process is entropically disfavoured when compared to 'normal' diazo-initiators and the C-N stretch vibrations are limited by π-conjugation. The formed diradical can either react with another TAD molecule to form a dimer (**61**) or expel carbon monoxide to form an isocyanate (**62**) (see Figure III.14).[38]

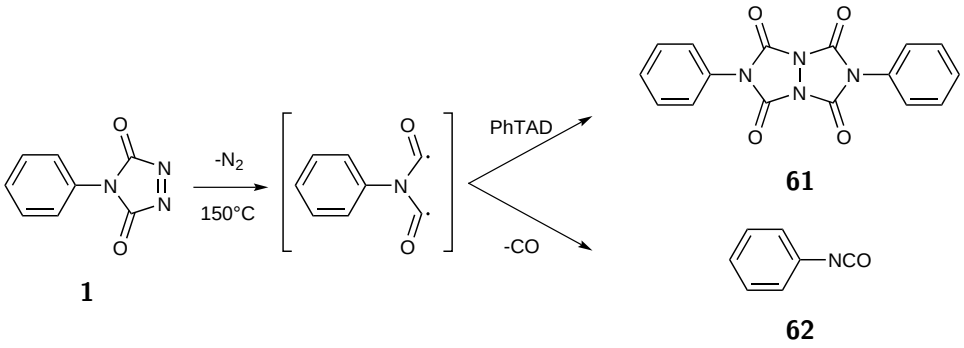


Figure III.14: Thermal decomposition of PhTAD (**1**) as described by Wamhoff.[38]

This behaviour can be verified by TGA measurements, that showed a very sharp decomposition peak of around 10 w% at 152 −153 °C. When isothermal TGA measurements were performed at different temperatures (see Figure III.15) the same conclusion could be observed (the small weight loss - around 5% at T <150 °C - was assigned to solvent loss, as confirmed by DSC analysis).

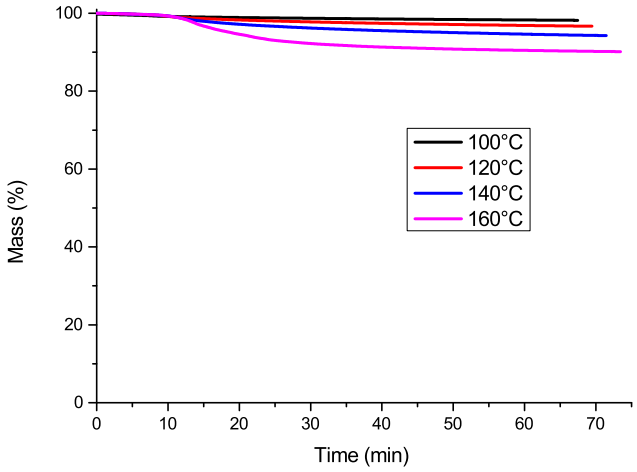


Figure III.15: Isothermal TGA curves of PhTAD (**1**).

Besides storage conditions, this thermal fragmentation process is also an important limitation of the use of TAD chemistry. Free TAD molecules cannot be exposed to temperatures higher than 150 °C in any circumstance. Luckily, due to the high reactivity of TAD, higher temperatures are usually not needed for the reaction. However if the specific application requires an increase of temperature, this can prove to be problematic.

III.5.2 Hydrolytic Stability

Lehn *et al.* investigated in detail what happened when TAD is exposed to a huge excess of water.[39] In case of aromatic TAD moieties, the aniline derivative (**63**) that is produced when PhTAD (**1**) decomposes (see Figure III.16a), can react with the remaining TAD in an electrophilic aromatic substitution (EAS) to form both a 1:1 (**64**) and a 1:2 adduct (**65**) (Figure III.16b). Due to this extra reaction the decomposition of TAD is accelerated in the case of aromatic TAD compounds. It is important to note that multiple TAD reactions in aqueous buffer solutions have been reported without significant side reactions.[40,41] This shows that the decomposition in water occurs rather slowly and the kinetic chemoselectivity again plays an important role.

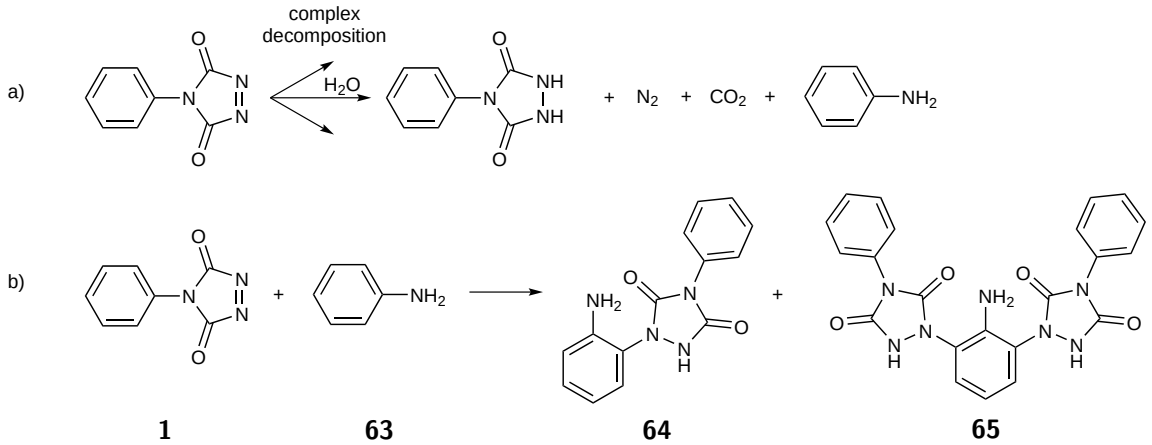
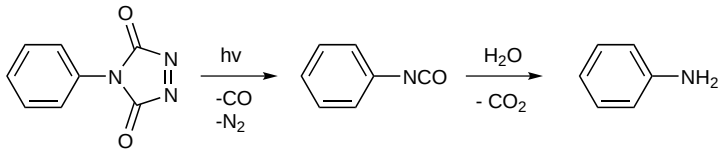


Figure III.16: (a) Main constituents of the complex decomposition of PhTAD (**1**) in the presence of water and (b) the possible cascade reaction of the formed aniline (**63**) with another PhTAD moiety.

III.5.3 Photolytic Stability

Additionally, the photolytic stability needs to be taken into account. Indeed, almost all reported synthesis routes advise to store the formed TAD in a dark environment.

Conversely, the possibility of photochemically induced reactions for specific applications can be investigated.

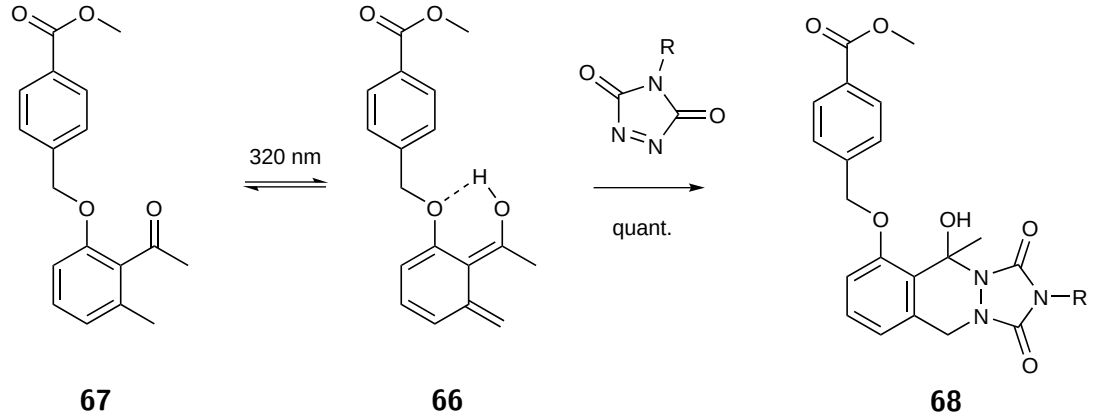


1 **62**

Figure III.17: Decomposition of PhTAD (**1**) under UV light.

Wamhoff and co-workers performed a complete study of the photolytic behaviour of PhTAD (**1**).[38] There it was shown that PhTAD (in acetonitrile, dichloromethane or benzene as solvent) decomposes to the corresponding isocyanate (**62**), with release of N_2 and CO, when irradiated with UV light ($\lambda > 313$ nm) – see Figure III.17. Besides this, it was noted that when aliphatic ethers were used as solvent, a side reaction between TAD and the solvent occurred, albeit in low yield. These results indicate a much higher reactivity of the TAD-triplet excited state.

Keeping in mind the *kinetic chemoselectivity*, a question arose whether TAD could react faster rather than it decomposes under UV-irradiation. For this a collaboration with the group of Prof. Barner-Kowollik (Karlsruhe, Germany), an expert in photochemical reactions, was started. During a two-month stay, TAD chemistry was combined with the photo-enol chemistry (developed in Karlsruhe).[42] With the aid of UV light, a highly reactive diene moiety (**66**) is created by isomerization of a 2-formyl-3-methylphenoxy (FMP, **67**) derivative. This diene can subsequently react with a TAD molecule to form a stable adduct (**68** - see Figure III.18).



67 **66** **68**

Figure III.18: Photo-enol chemistry combined with TAD.

In a first series of experiments, the aim was to check if TAD components were actually suitable to be used in UV experiments. For this a solution of a small molecular weight TAD component (BuTAD) in acetonitrile was irradiated for one hour at different wavelengths (see Figure III.19). Before and after each experiment, ^{1}H NMR spectra were taken to check for possible side reactions or decomposition. However in almost all experiments no side or decomposition products were observed making it possible to use TAD chemistry in combination with UV triggered systems with favourable kinetics.

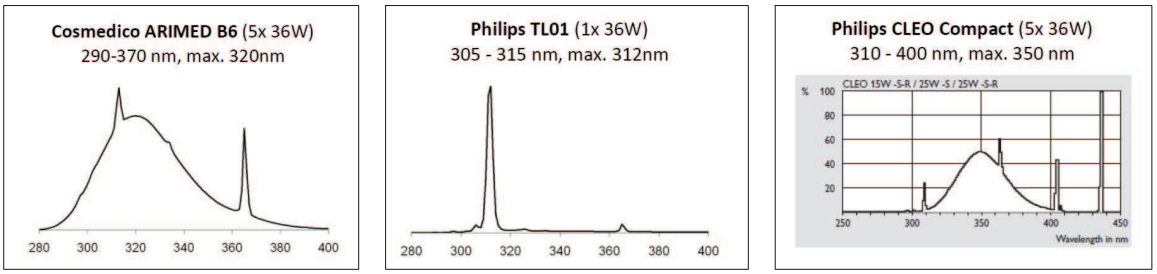


Figure III.19: UV-spectra of the lamps used.

To determine the efficiency of the reaction between TAD and a photo-enol, an easy to characterize model reaction between BuTAD and FMP was performed. As reaction conditions, 5 mg/mL in acetonitrile – 1:1 ratio, was chosen. This led to a complete conversion to the desired adduct in 50 minutes – as determined by ESI-MS (see Figure III.20). Surprisingly, there was no difference between the different UV-sources, they all had a comparable kinetic behaviour with similar conversion rates (approx. 50 minutes).

This conversion was already a nice *proof-of-concept* that the combination TAD-photo-enol works. However, since the rate determining step in this process is the isomerisation of FMP and not the DA reaction itself, no distinct difference with other dienophiles (e.g. maleimides) was detected.[43–45] In order to have a greater effect, the concentration was decreased to 1 mg/mL. In this case, the TAD reaction went to full conversion in 20 minutes, while remaining 40 minutes for maleimides. This distinct kinetic acceleration is much larger for TAD than for other dienophiles making this a nice starting point for future investigations. In light of these promising first results, a joint PhD project (Hannes Houck) was started up between the two groups. In this way the possible use of TAD in combination with UV can be explored more in detail as 'light-triggered' *click* reactions.

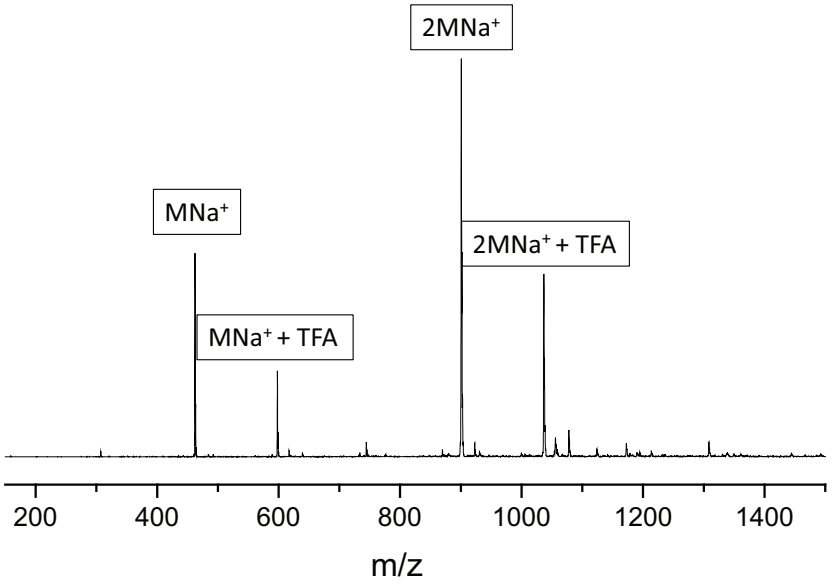


Figure III.20: ESI-MS spectra of reaction between BuTAD (**31**) and FMP-COOMe (**67**) after 50 minutes irradiation by Arimed B6.

III.5.4 Solvent compatibility

Going more into detail, the effect of solvents on TAD-containing molecules was investigated. Table III.6 gives an overview of solvents that have a reported (side) reaction with TAD, in combination with solvents that, in our hands, are safe to be used with TAD molecules.

Of course, not having reported side reactions with TAD does not mean that this is an ideal solvent to be used. In the case of the aromatic solvents (benzene, toluene) TAD most likely will not dissolve and therefore such solvents can only be used as a co-solvent, if needed. Important to note is the fact that when electron-rich aromatics are used, side reactions still can occur (as was described in section II.3.3). In this respect, it has to be mentioned that a lot of different solvents can be used in combination with TAD moieties (water, alcohols...) as long as the kinetic behaviour of the wanted reaction is better than the side reaction. To the best of our knowledge, acetonitrile has the best solvating power for TAD molecules, followed by chlorinated solvents (DCM, chloroform).

Table III.6: Solvent stability for TAD molecules - green '∨' (no reported side reactions), yellow '-' (no reported side reactions but difficult to dissolve TAD) and red '×' (reported side reactions).

Solvent	Stability	Ref.	Solvent	Stability	Ref.
Acids	×	7	DMF	×	46
Acetonitrile	✓	-	DMSO	×	7
Alcohols	×	7,29,47,48	Ethers	×	6,29,38
Anisole	×	38	Esters	✓	-
Bases	×	46,47	Ketones	×	6,7,39,49,50
Benzene	-	-	Toluene	-	-
Chlorinated	✓	-	Water	×	38,39,48

III.5.5 Storage tests

In this work, also an investigation of suitable storage conditions was performed. Thus, freshly oxidized BuTAD (**31**) was prepared and stored at different conditions. On a regular basis, a ^{1}H NMR measurement was performed to check for decomposition. Storage was performed both in solution ($CDCl_3$ and DMSO-d_6) and in powder form at different temperatures (−18 ,4 and 25 °C). Important to note is that storage was conducted in a dark environment (brown flask) to avoid possible photolytic degradation.[7]

From these tests, it is clear that TAD molecules must be stored at lower temperatures (−18 °C) (see Table III.7), in order to avoid thermal decomposition and to retard possible side reactions (hydrolysis). Deuterated solvents were chosen to simplify characterization and to limit water contamination. Still, due to the fact that there is some water present in those solvents, hydrolysis occurs (even at lower temperature) in solution.

Table III.7: Storage test of BuTAD (**31**) at different conditions in a dark environment shown as the maximum number of days BuTAD is considered to be stable. The threshold for stability was set at 5% of degradation, verified via ^{1}H NMR.

(days)	−18 °C	4 °C	25 °C
Powder	+30	6	3
$CDCl_3$ solution	21	14	10
DMSO-d_6 solution	20	12	8

Based on these results TAD molecules are best stored at −18 °C as a powder away from light (brown flask). Tests show that TAD molecules could be stored for over one month without any detectable side reactions. If the possibility exists to sublimate the TAD molecule, the compounds can even be stored up to one year.[51] A crucial point to make is that the precursor of TAD, urazole, is far more stable than its corresponding TAD derivative. Hence, urazole derivatives can already be stored under dry conditions at room temperature. Within the time frame of this project, no decomposition was observed for urazole moieties when stored (period of more than three years).

III.6 Toxicity of TAD-related compounds

Although this topic is far beyond the scope of this doctoral work, some basic information concerning the toxicity is necessary before one can start exploiting TAD chemistry in applications that can expose organisms or the environment to TADs and/or urazoles. Little is known about TAD components regarding toxicity, however this does not pose an immediate threat concerning future applications. It will not come as a surprise that, due to the high reactivity with a whole range of components, the pure TAD moieties will most likely not be used as such in consumer end products. For this reason the toxicity question shifts away from TAD towards urazoles (as starting product and/or adduct). The presence of a quite acidic proton on the formed urazole can be problematic and needs to be investigated in more detail.

For this reason a slug mucosal irritation (SMI) study was performed on 4-phenyl- and 4-butylurazole (PhUr – BUr). The SMI assay[52] is developed at the Laboratory of Pharmaceutical Technology (UGent) to predict the stinging, itching or burning potential of the test items (in this case the involved urazoles). The production of mucus of the slug can be translated to both ocular and nasal (human) discomfort. From these tests, there could be concluded that even at 10% w/v of either BUr or PhUr no discomfort is to be expected. This good news was the starting point to check in literature whether urazoles were already implemented in pharmaceutical applications. Selected examples of this were already discussed in section II.5.1.

Additionally, it was found that some urazole derivatives show potent cytotoxic behaviour in murrain and human cancer cell lines and also have a significant inhibition effect on DNA synthesis (with moderate inhibition in RNA synthesis).[53] The main reason for this cytotoxicity is probably the inhibition of rate limiting enzymes, as suggested by kinetic studies.[54] Additional pharmaceutical properties were also found[55] ranging from hypolipidemic activity[55,56], herbicides[57,58], pesticides[59] and insecticides[60,61]. The fact that some patents have appeared claiming pharmaceutical applications of various urazole derivatives can be taken as a sign that no obvious general toxicity mechanisms are associated with urazole compounds as such.

To conclude, urazoles (and derivatives thereof) have interesting characteristics that have already been employed in pharmaceutical applications (*vide infra*). However, more research questions need to be answered to provide a full understanding of their toxicity. Collaboration with the *Biopharmaceutical Technology Unit* led by Prof. Bruno De Geest will surely add to this matter (started in the spring of 2015).

III.7 Conclusions and perspectives

While introducing a new chemical platform, it is crucial to have a good overview of all known and relevant characteristics of the molecules involved. Since most of the literature is focused on TAD reactivity and there is no clear overview of the chemical properties, this chapter tried to give a summary of all the relevant features that until today were not studied systematically.

In a first part, the orthogonal behaviour of TAD chemistry in both DA and reversible AE was studied. In this respect, a range of different additives (containing functional groups) were added to the starting mixture in order to determine which functionalities can be present without interfering the reaction. It became clear that only amines should be avoided in order to get a clean reaction.

Once it was established which functionalities can be tolerated, different functional compounds were prepared, in this way the lack of commercially available TAD components could be addressed. It has to be noted that urazole and not TAD components were prepared, due to the superior stability of the former.

Chapter II described the reactivity of TAD, while here a summary was made of all relevant rate constants of known DA and AE reactions. The kinetic hierarchy gave the ability to predict the main reaction product in more complex reaction media. Additionally, the reaction rate of TAD differs greatly (up to 8 orders of magnitude difference in rate constants) by varying the complementary partner. Also, the stability of TAD and its adducts was explored, leading to the optimal storage conditions.

Finally, the toxicity of TADs was shortly discussed.

This chapter gave an overview of the relevant properties of TAD chemistry, crucial for every project adopting this new platform. Some interesting insights were obtained (concerning stability, toxicity...) leading to new projects within our group.

III.8 Experimental section

III.8.1 Materials

6-Aminocaproic acid (99%), anisole (99%), benzyl alcohol (99.8%), benzyl bromide (98%), benzyl chloroformate (>98%), 4-benzyloxybutyric acid (95%), bromine (reagent grade), 1-bromobutane (99%), α-bromoisobutyryl bromide (98%), butylamine (99.5%), n-butyllithium (2.5 M in hexane), ϵ-caprolactone (97%), celite®, 1,4-diazabicyclo[2.2.2]octane (DABCO, 99%), *N*,*N'*-dicyclohexylcarbodiimide (DCC, 99%), 4-(dimethylamino)pyridine (DMAP, >99%), 1-dodecanethiol (>98%), N-ethyl maleimide (>98%), furan (>99%), glycidol (96%), *trans*,*trans*,2,4-hexadien-1-ol (>97%), 1-hexyne (97%), N-hydroxysuccinimide (NHS, 98%), N-methylacetamide (>99%), methyl acrylate (99%), 3-methylbutyraldehyde (97%), methyl methacrylate (99%), 4-nitrophenyl isocyanate (97%), 1-octene (98%), palladium hydroxide (5% on activated carbon), palladium on carbon (5%), 2-phenyl-acetophenone (97%), phenylhydrazine (97%), potassium hydroxide (reagent grade, 90%, flakes), propionic acid (>99.5%), propionitrile (99%), propiophenone (99%), sulphuric acid (>99%), trifluoro acetic acid (99%), trimethylacetylchloride (99%) and o-toluidine (98%) were purchased from Sigma-Aldrich. Butyl isocyanate (>98%) and diphenylphosphoryl azide (>96%) from TCI, hydrochloric acid (36%) with Chem-Lab. Ammonium chloride (99%), potassium carbonate (99%) and sodium bicarbonate (99.5%) from Roth. Hexamethylene diisocyanate (>98%) with Fluka, magnesium sulphate (anhydrous) from Boom. Ethyl carbazate (97%), hydrochloric acid (4N, in dioxane) and sodium hydroxide (97%) from Acros Organics. All solvents (Sigma-Aldrich) and products were used without any pre-treatment or purification.

III.8.2 Characterization

Nuclear Magnetic Resonance (NMR) ^{1}H-spectra were recorded with a Bruker Avance 300 (300 MHz) FT-NMR spectrometer in $CDCl_3$ (Eurisotop) or DMSO-d_6 solution at room temperature. Chemical shifts are presented in parts per million (δ) relative to $CHCl_3$ (7.26 ppm for ^{1}H-NMR) and DMSO (2.50 ppm for ^{1}H-NMR) as an internal standard. The resonance multiplicities are descried as [br. (broad)] s (singlet), d (doublet), t (triplet), q (quadruplet), quin (quintuplet), sext (sextuplet) or m (multiplet).

Thermogravimetric analysis (TGA) was performed using a Mettler-Toledo TGA / SDTA 851e apparatus. Samples (5 to 10 mg) were heated in a nitrogen atmosphere with a heating rate of 10 K min^{-1} going from 25 °C to 600 °C. For the analysis of the thermograms, the STARe software of Mettler-Toledo was used.

Electrospray Ionization Mass Spectrometry (ESI-MS) Measurements were recorded on an LXQ mass spectrometer (ThermoFisher Scientific, San Jose, CA) equipped with an atmospheric pressure ionization source operating in the nebuliser assisted electrospray mode. The instrument was calibrated in the m/z range of 195–1822 using a standard containing caffeine, Met-Arg-Phe-Ala acetate (MRFA) and a mixture of fluorinated phosphazenes (Ultramark 1621) (all from Aldrich). A constant spray voltage of 3.5 kV and a dimensionless sheath gas of 8 and a sweep gas flow rate of 2 were applied. The capillary voltage, the tube lens offset voltage, and the capillary temperature, were set to 60 V, 120 V, and 275 °C, respectively.

UV-Vis measurements were performed on an AnalytikJEna Specord 200 in quartz cuvettes with a thickness of 10 mm in a wavelength range of 200 to 700 nm. The concentration of each sample was 1 mg/mL.

Stopped-flow UV measurements are performed by rapidly inserting small volumes of solutions into a high efficiency mixer to initiate a fast reaction. The resulting mixture is then guided into a UV-observation cell where the injected volume is limited by the stop syringe which provides the 'stopped-flow'. The solution entering the flow cell is only milliseconds old (= ´dead time' of the stopped-flow system). As the solution fills the stopping syringe, the plunger hits a block, causing the flow to be stopped instantaneously, making it possible to measure the kinetics of the reaction in the UV-cell which then decays in the cells starting from a reproducible and completely homogeneous steady-state regime (see Figure III.21). All measurements were performed by Laetitia Vlaminck during a research stay in the group of E.W. Meijer (Eindhoven University of Technology).

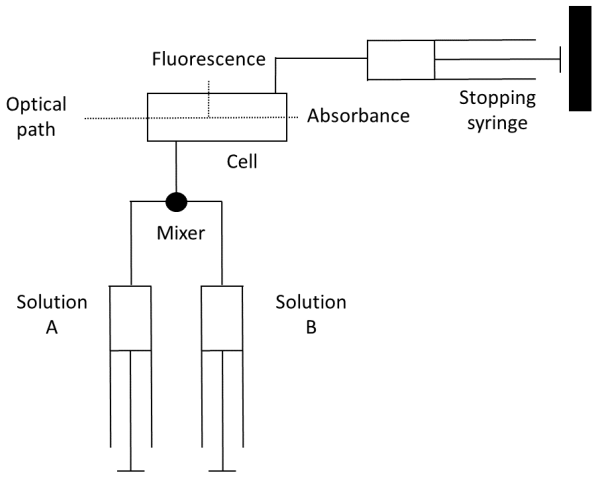
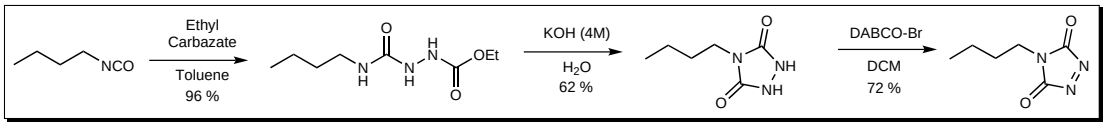


Figure III.21: Stopped-flow measurement.

III.8.3 Synthesis

III.8.3.1 4-Butyl-1,2,4-triazoline-3,5-dione (BuTAD, 31)



A mixture of ethyl carbazate (10 g, 96.1 mmol, 1 equivalent) and toluene (105 mL) was placed in a three neck flask (250 mL) and cooled in an ice bath. The flask was equipped with an addition funnel, containing butyl isocyanate (10.8 mL, 96.1 mmol, 1 equivalent), a mechanical stirrer and a bulb condenser. The mixture was put under inert atmosphere and the isocyanate was added slowly under vigorous stirring. After addition the mixture was stirred at room temperature for two hours, followed by two hours at 90 °C. After cooling down the reaction to room temperature, 4-butyl-1-(ethoxycarbonyl) semicarbazide was filtered off and washed with toluene (18.75 g, 96%).

Then in a 50 mL flask, 4-butyl-1-(ethoxycarbonyl) semicarbazide (12.2 g, 60.0 mmol) was dissolved in 30 mL of an aqueous potassium hydroxide solution (KOH- 4M) under inert atmosphere. This mixture was refluxed for 1.5 hour (100°C), warm filtered, cooled to room temperature and acidified until pH 1 due to addition of hydrogen chloride. This mixture was cooled to room temperature to yield a solid white powder that was filtered off (5.85 g, 62%).

In a last step, a mixture of 4-butyl-1,2,4-triazolidine-3,5-dione (1 g, 6.36 mmol, 1 equival-

ent), DABCO-Br (**7**, 2 g, 1.27 mmol, 0.2 equivalent) and dichloromethane (30 mL) was put in a flask (100 mL) under inert atmosphere and stirred for two hours at room temperature. The solids were filtered off and washed with dichloromethane (2 × 30 mL). The obtained filtrate was concentrated *in vacuo* obtaining 4-butyl-1,2,4-triazoline-3,5-dione as pure compound (0.71 g, 72%).[i]

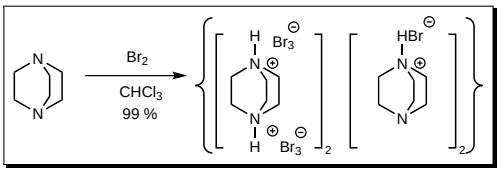
Bruto formula: $C_6H_9N_3O_2$.

MW.: 155.15 g/mol.

^{1}H-NMR (300 MHz, DMSO−d_6): δ (ppm) = 0.88 (t, 3 H, CH_3−(CH$_2$)$_3$), 1.30 (m, 2 H, CH$_3$−CH_2−CH$_2$), 1.56 (m, 2 H, N−CH$_2$−CH_2), 3.47 (t, 2 H, N−CH_2−CH$_2$).

Reference: R. C. Cookson, S. S. Gupte, I. D. R. Stevens, C. T. Watts, Organic Syntheses, 1988, 50-9, 936–940.

III.8.3.2 1,4-diazabicyclo[2.2.2]octane bromide complex(DABCO-Br, 7)



In a 500 mL two-neck flask, 1,4-diazabicyclo[2.2.2]octane (6.73 g, 60.0 mmol, 1 equivalent) was dissolved in chloroform (100 mL). In a next step, a solution of bromine (20.0 g, 0.125 mol, 2.1 equivalents) in chloroform (100 mL) was added dropwise using an addition funnel. The resulting mixture was stirred under inert atmosphere for one hour (for adequate mixing, an oval shaped, slightly oversized stirring bar was used). The yellow precipitate was filtered off, washed with chloroform (50 mL) and dried overnight in a vacuum oven at 40 °C. DABCO-Br (23.3g - 99%) was obtained free from contaminents and used as such in the next step.

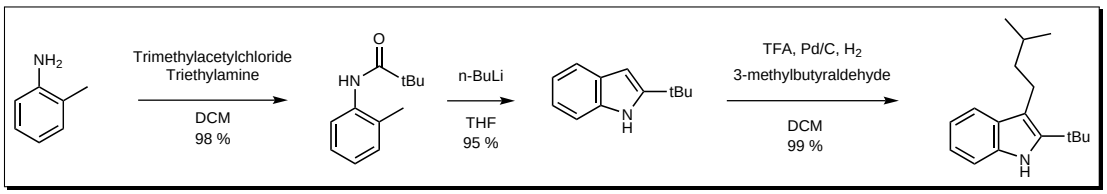
Bruto formula: $C_{24}H_{54}Br_{14}N_8$.

MW.: 1573.40 g/mol.

Reference: M. M. Heravi, F. Derikvand, M. Ghassemzadeh, B. Neumuller, Tetrahedron Letters, 2005, 46, 6243–6245.

[i]The temperature of the heating bath cannot exceed 50 °C due to the volatility of the obtained compound.

III.8.3.3 2-*tert*-butyl-3-*iso*pentyl-1*H*-indole (34)



In a 500 mL flask *o*-toluidine (30.0 g, 0.280 mol, 1 equivalent) was dissolved in dichloromethane (240 mL). After addition of triethylamine (31.2 g, 0.308 mol, 1.1 equivalent), the solution was cooled with a water bath and trimethylacetylchloride (37.9 mL, 0.308 mol, 1.1 equivalent) was added dropwise. This mixture was stirred overnight under inert atmosphere. The reaction mixture was then washed with an aqueous hydrogen chloride solution (360 mL, 5%), saturated sodium carbonate solution (360 mL) and brine (360 mL). After drying on magnesium sulfate N-*o*-tolylpivalamide was obtained after evaporating of all the organic solvents under reduced pressure (52.4 ivory white powder – 98%).

Next, in a 1 L flask a solution of N-*o*-tolylpivalamide (35g, 0.183 mol, 1 equivalent) in anhydrous tetrahydrofuran (THF, 120 mL) is prepared under inert atmosphere. After cooling down in a water bath *n*-butyllithium (2.5 M in hexanes, 220 mL, 0.55 mol, 3 equivalents) was added dropwise. The reaction mixture was stirred overnight at room temperature, after which it was cooled in an ice bath. Then, saturated aqueous ammonium chloride solution (340 mL) was added slowly. The water phase was extracted with ethyl acetate (2 x 340 mL), after which the combined organic phases were dried on magnesium sulphate, filtered and concentrated *in vacuo* (30.0 g brown solid – 95%).

In a last step, a mixture of trifluoro acetic acid (3.42 g, 30 mmol, 1.5 equivalent), palladium (5% on activated carbon, 0.3 g) and dichloromethane (40 mL) was treated with hydrogen gas in a 250 mL two neck flask while cooling with an ice bath. To this mixture, 2-*tert*-butyl-1*H*-indole (3.46 g, 20 mmol, 1 equivalent) and 3-methylbutyraldehyde (1.9 g, 22 mmol, 1.1 equivalent) in dichloromethane (60 mL) was added dropwise. This solution was stirred in a water bath for five hours, regularly flushing with hydrogen gas. With the help of TLC (hexane:ethyl acetate 9:1) the reaction was followed until completion. The mixture was filtered over celite and washed with saturated aqueous sodium carbonate solution (100 mL). The combined organic phases were dried over magnesium sulphate and concentrated *in vacuo* to obtain the title compound (4.83 g – 99%).

Bruto formula: $C_{17}H_{25}N$.

MW.: 243.39 g/mol.

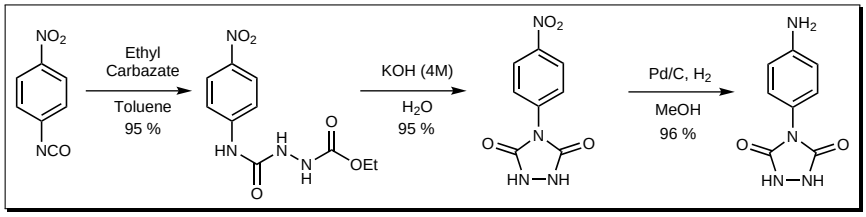
HRMS (m/z for [MH]+): calculated: 244.2060; experimental: 244.2067.

^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) = 0.93 (d, 6 H, $CH(CH_3)_2$), 1.37 (s, 9 H, $C(CH_3)_3$)), 1.47 (m, 2 H, i−Pr−CH_2), 1.65 (m, 1 H, CH−$(CH_3)_2$), 2.76 (m, 2 H, C= C−CH_2), 7.00 (m, 2 H, ArH), 7.18 (d, 1 H, ArH), 7.42 (d, 1 H, ArH), 7.70 (br.s, 1 H, NH).

^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) = 22.69 (CH_3), 23.43 (CH_2), 28.87 (CH), 30.59 (CH_3), 32.95 (C), 40.81 (CH_2), 110.33 (CH), 111.36 (C), 118.16 (CH), 119.94 (CH), 120.97 (CH), 129.83 (C), 134.07 (C), 141.16 (C)

Reference: L. L. Cao, D. S.Wang, G. F. Jiang, Y. G. Zhou, Tetrahedron Letters 2011, 52, 2837–2839.

III.8.3.4 Aniline urazole (39)



In a 50 mL two-neck flask, 4-nitrophenyl isocyanate (1 g, 6.1 mmol) was dissolved in dry toluene (15 mL). This mixture was cooled on an ice bath for 30 min. Then a solution of ethyl carbazate (0.634 g, 6.1 mmol) in anhydrous toluene (15 mL) was added dropwise over a period of 10 minutes. The reaction mixture was brought to room temperature and allowed to stir for 8 hours. After cool down (ice bath), the desired product was filtered off and used in the next step without any further purification (1.56 g bright yellow powder – 95%).

For the next stage a 5 mL flask was charged with 4-nitrophenyl 1-(ethoxycarbonyl) semicarbazide (1 g, 3.7 mmol) toghether with an aqueous potassium hydroxide solution (2.5 mL, 4M) under inert atmosphere. This resulting mixture was refluxed for four hours (oil bath at 100 °C). The solution was filtered off warm, cooled to room temperature and acidified until pH 1 using hydrogen chloride. This mixture was cooled to room temperature yielding a solid white powder that was filtered off (0.77 g – 95%).

In a 25 mL flask, 4-nitrophenyl 1,2,4-triazolidine-3,5-dione (0.5 g, 2.6 mmol) was dissolved in methanol (10 mL). Then, palladium (5% on activated carbon) was added. The set-up was equipped with a balloon filled with hydrogen. First the system was put under vacuum to remove the inert atmosphere, then the tap of the hydrogen balloon was opened. This procedure was repeated four times to ensure full saturation with hydrogen gas. This mixture was stirred vigorously for 24 hours at room temperature. The solution was filtered over a plug of celite and concentrated under reduced pressure giving aniline urazole (0.48 g – 96%).

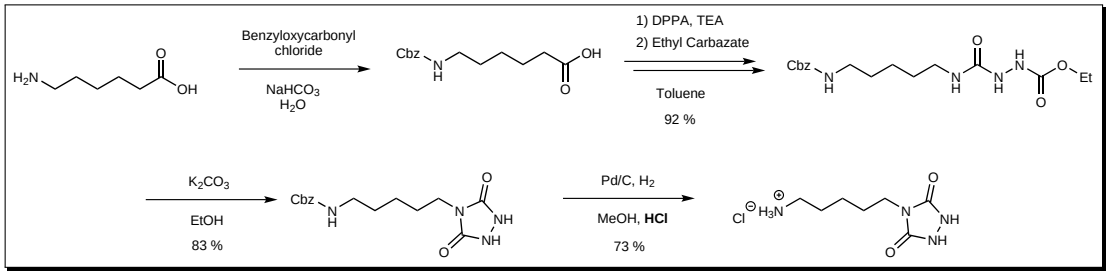
Bruto formula: $C_8H_8N_4O_2$.

MW.: 192.17 g/mol.

^{1}H-NMR (300 MHz, DMSO−d_6): δ (ppm) = 5.29 (br.s, 2 H, NH_2), 6.59 (d, 2 H, ArH), 6.98 (d, 2 H, ArH), 10.17 (br.s, 2 H, NH).

Reference: S. E. Mallakpour, H. Nasr-Isfahani, Indian Journal of Chemistry, 2002, 41B, 169–174.

III.8.3.5 4-(5-Aminopentyl)-1,2,4-triazolidine-3,5-dione (44)



In a 100 mL flask 6-aminocaproic acid (1.81 g, 13.8 mmol, 1 equivalent) was mixed with sodium bicarbonate (1.73 g, 20.6 mmol) and dissolved in water (20 mL). This solution was cooled in an ice bath and benzyloxycarbonyl chloride (2.4 mL, 16.8 mmol, 1.2 equivalent) was added dropwise. The reaction was stirred for 18 hours at room temperature, filtered and washed with hexane. After drying, 6-benzyl carbamate caproic acid was obtained and used without any further purification in the next step.

The obtained Cbz-protected derivative (2.42 g, 9.12 mmol, 1 equivalent) was dissolved in 60 mL toluene and to this triethylamine (2,5 mL, 18.0 mmol, 2 equivalents) and diphenylphosphoryl azide (DPPA, 2 mL, 9.12 mmol, 1 equivalent) was added. The reaction was

placed under inert atmosphere and equipped with a reflux condenser, followed by stirring for one hour at room temperature and 6 hours at 110 °C. After this the reaction was cooled to room temperature and ethyl carbazate was added (1 g, 9.12 mmol, 1 equivalent). The reaction was stirred overnight at room temperature followed by evaporating under reduced pressure in the presence of silica (5 g). Flash chromatography (with dry loading, eluent = ethyl acetate)(rf = 0.5) was used to yield to pure semi-carbazide.

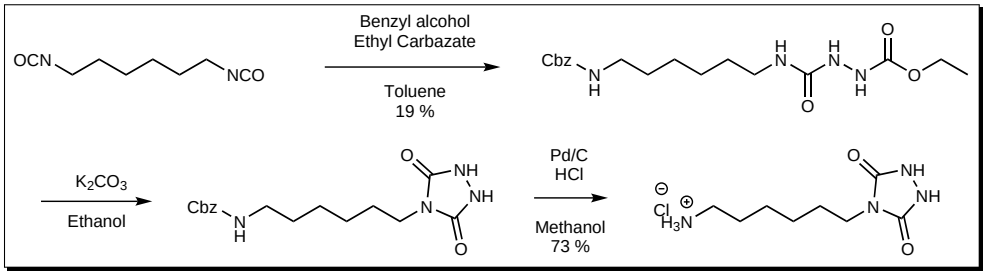
The basic ring closure of the semi-carbazide was performed by dissolving semi-carbazide (1.0 g, 2.72 mmol, 1 equivalent) in ethanol (11 mL). To this potassium carbonate (1.5 g, 10.9 mmol, 4 equivalents) was added and the reaction was equipped with a reflux condenser. The mixture was stirred for 16 hours at 65 °C. Afterwards, the reaction was cooled to room temperature and filtered. The filtrate was acidified to pH = 4 using hydrochloric acid (4N, in dioxane), filtered and concentrated under reduced pressure in the presence of a 5-fold excess of silica. Flash chromatography (with dry loading) is performed to purify the urazole (eluent = ethyl acetate:heptane 2:3 to 100 % ethyl acetate)(rf = 0.26).

In a last step the Cbz group is removed by dissolving the urazole (0.5 g, 1.5 mmol) in methanol (10 mL). A catalytic amount of palladium (5% on activated carbon) and hydrochloric acid (2 equivalents) were added. Then a balloon containing hydrogen gas was placed on the reaction, this mixture was stirred vigorously for 2 hours at room temperature. The solution was filtered over a syringe filter to remove the palladium and was then concentrated under reduced pressure to give the aliphatic amine urazole (0.25 g – 73%).

Bruto formula: $C_7H_{15}ClN_4O_2$.
MW.: 222.67 g/mol.
^{1}H-NMR (300 MHz, DMSO−d_6): δ (ppm) = 1.29 (m, 2 H, CH_2−CH_2−CH_2), 1.56 (m, 4 H, 2 x CH_2), 2.77 (m, 2 H, CH_2−NH_3), 3.35 (t, 2 H, CH_2−N), 7.80 (br.s, 3 H, NH_3), 10.08 (s, 2 H, NH).

Reference: G. Read, N.R. Richardson, Journal of the Chemical Society, Perkin Transactions 1, 1996, 167-174.

III.8.3.6 4-(6-Aminohexyl)-1,2,4-triazolidine-3,5-dione hydrochloride (44)



In a 500 mL flask a mixture of benzyl alcohol (16.22 g, 0.15 mol, 1 equivalent) and ethyl carbazate (15.62g, 0.15 mol, 1 equivalent) in anhydrous toluene (200 mL) was added over 45 minutes to a stirred solution of hexamethylene diisocyanate (25.23 g, 0.15 mol, 1 equivalent) in anhydrous toluene (50 mL) under nitrogen atmosphere maintained at 0 −5 °C. Afterwards, the solution was allowed to warm up to room temperature, refluxed for 1.5 hours and left to stand overnight. The waxy solid was filtered and washed with boiling hexane (3 x 50 mL) to give a white powder (4,4'-hexane-1,6-bis-semi-carbazide, 19.5 g – 33%). The filtrate was evaporated and re-dissolved in ethyl acetate. Flash chromatography was conducted (eluens= (ethyl acetate -> ethyl acetate:MeOH; 6:1) to yield firstly N,N'-bis(benzyloxycarbonyl)hexane (19.2 – 30% - rf = 0.73) and secondly the desired product (10.26 g – 19%).

The basic ring closure of the semi-carbazide was performed by dissolving the semi-carbazide (1.0 g, 2.62 mmol, 1 equivalent) in ethanol (11 mL). To this potassium carbonate (1.45 g, 10.5 mmol, 4 equivalents) was added and a reflux condenser was fitted. The mixture was stirred for 16 hours at 65 °C. Afterwards, the reaction was cooled to room temperature and filtered. The filtrate was acidified to pH = 4 using hydrochloric acid (4N, in dioxane), filtered and concentrated under reduced pressure in the presence of a 5-fold excess of silica. Flash chromatography (with dry loading) was performed to purify the urazole (eluent = ethyl acetate:heptane 2:3 to 100 % ethyl acetate)(rf = 0.25).

In a last step the Cbz group was removed by dissolving the urazole (0.5 g, 1.5 mmol, 1 equivalent) in 10 mL of methanol. A catalytic amount of palladium (5% on activated carbon) and hydrochloric acid (73 µL, 37%, 2 equivalents) were added. Then a balloon containing hydrogen gas was installed and stirring was continued vigorously for two hours at room temperature. The solution was filtered over a syringe filter to remove the palla-

dium and then concentrated under reduced pressure to give the desired product (0.25 g – 73%).

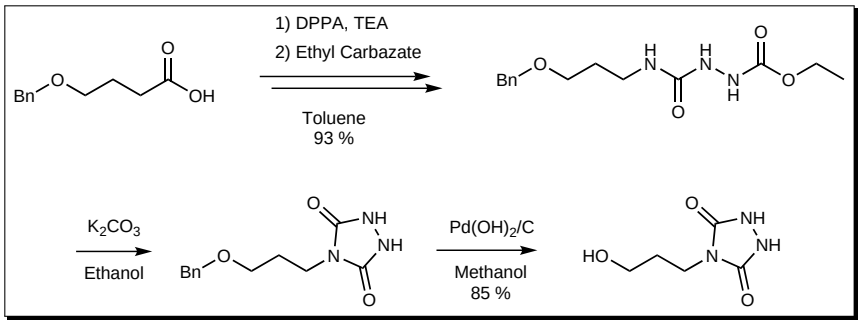
Bruto formula: $C_8H_{17}ClN_4O_2$.

MW.: 236.70 g/mol.

^{1}H-NMR (300 MHz, DMSO−d_6): δ (ppm) = 1.30 (band, 4 H, CH_2−CH_2−CH_2−CH_2), 1.53 (band, 4 H, 2 xCH_2), 2.73 (m, 2 H, CH_2−NH_3), 3.35 (t, 2 H, CH_2−N), 7.87 (br.s, 3 H, NH_3), 10.08 (s, 2 H, NH).

Reference: G. Read, N.R. Richardson, Journal of the Chemical Society, Perkin Transactions 1, 1996, 167-174.

III.8.3.7 4-(3-Hydroxypropyl)-1,2,4-triazolidine-3,5-dione (51)



4-benzyloxybutyric acid (1 g, 5.15 mmol, 1 equivalent) was dissolved in toluene (30 mL) and to this triethylamine (1.44 mL, 10.3 mmol, 2 equivalents) and diphenylphosphoryl azide (DPPA, 1.11 mL, 5.15 mmol, 1 equivalent) was added. The reaction was placed under inert atmosphere and equipped with a reflux condenser, followed by stirring for one hour at room temperature and 6 hours at 110 °C. After this the reaction was cooled to room temperature and ethyl carbazate was added (0.54 g, 5.15 mmol, 1 equivalent). The reaction was stirred overnight at room temperature followed by concentrating under reduced pressure. Flash chromatography (eluent = ethyl acetate)(rf = 0.3) was used to yield to pure semi-carbazide.

The basic ring closure of the semi-carbazide was performed by dissolving semi-carbazide (1.5 g, 5 mmol, 1 equivalent) in ethanol (20 mL). To this potassium carbonate (3 g, 20 mmol, 4 equivalents) was added and the reaction was equipped with a reflux condenser. The mixture was stirred for 16 hours at 65 °C. Afterwards the reaction was cooled to room

temperature and filtered. The filtrate was acidified to pH 4 with the aid of hydrochloric acid (4N, in dioxane), filtered and concentrated under reduced pressure in the presence of a 5-fold excess of silica. Flash chromatography (with dry loading) was performed to purify the urazole (eluent = ethyl acetate:heptane 2:3 to 100 % ethyl acetate)(rf = 0.2).

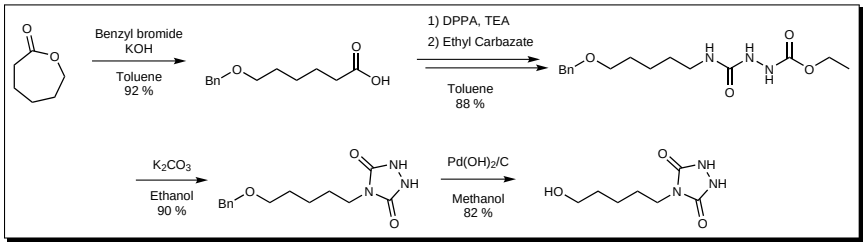
In a last step the benzyl ether was removed by dissolving the urazole (0.5 g, 2 mmol) in methanol (10 mL). Palladium hydroxide (5% on activated carbon) was added. Then, a balloon containing hydrogen gas was placed on the reaction, this mixture was stirred vigorously for two hours at room temperature. The solution was filtered over a syringe filter to remove the palladium hydroxide and then concentrated under reduced pressure to give the desired product (0.27 g – 85%).

Bruto formula: $C_5H_9N_3O_3$.

MW.: 159.15 g/mol.

^{1}H-NMR (300 MHz, DMSO$-$d$_6$): δ (ppm) = 1.66 (q, 2 H, $CH_2-CH_2-CH_2$), 3.40 (2 x t, 2 x 2 H, $CH_2-CH_2-CH_2$), 10.01 (s, 2 H, NH).

III.8.3.8 4-(5-Hydroxypentyl)-1,2,4-triazolidine-3,5-dione (55)



Freshly crushed potassium hydroxide (85 %, 49.2 g, 0.745 mol) was added to a solution of benzyl bromide (120 g, 0.7 mol) and ϵ-caprolactone (20.06 g, 0.176 mol) in toluene (300 mL) at room temperature. The mixture was heated at reflux for 48 hours using a Dean-Stark apparatus. Then the mixture was diluted with diethylether (200 mL) and washed with H_2O (300 mL). The aqueous layer was extracted twice with diethylether (200 mL). The combined organic layer was concentrated *in vacuo* to 150 mL and was set aside. The aqueous layer was cooled in an ice bath and an aqueous solution of sulphuric acid (2M, 180 mL) was added. The turbid aqueous solution was extracted with dichloromethane (3 x 200 mL), dried over anhydrous magnesium sulphate and concentrated in vacuum to afford 6-(benzyloxy)hexanoic acid as a pale yellow oil. To the residue of the toluene/diethylether

layers, sodium hydroxide pellets (16 g, 0.4 mol) and H_2O (80 mL) were added. The resulting mixture was heated at reflux temperature for 24 hours. The aqueous layer was separated, diluted to 250 mL with water and washed with diethylether (3 x 100 mL). The aqueous layer was acidified with a slurry of concentrated sulphuric acid (25 mL) in ice (100 mL) and extracted with dichlorometane (3 x 100 mL). The organic layers were dried over anhydrous magnesium sulphate and the solvent was removed in vacuum to afford 6-(benzyloxy)hexanoic acid as a pale yellow oil (36.3 g, 0.16 mmol, 92.7%).

6-(benzyloxy)hexanoic acid (1 g, 4.5 mmol) was dissolved in toluene (30 mL) and to this triethylamine (1.25 mL, 9 mmol) and diphenylphosphoryl azide (DPPA, 0.97 mL, 4.5 mmol) was added. The reaction is placed under inert atmosphere and equipped with a reflux condenser, followed by stirring for 1 hour at room temperature and 6 hours at 110 °C. Afterwards, the reaction was cooled to room temperature and ethyl carbazate is added (0.47 g, 4.5 mmol). The reaction was stirred overnight at room temperature and concentrated under reduced pressure. Flash chromatography (eluent = ethyl acetate)(rf = 0.26) was used to yield to pure semi-carbazide .

The basic ring closure of the semi-carbazide was performed by dissolving the semi-carbazide (1.5 g, 4.6 mmol) in ethanol (20 mL). Potassium carbonate (2.56 g, 18.5 mmol) was added and a reflux condenser was installed. The mixture was stirred for 16 hours at 65 °C. Afterwards, the reaction was cooled to room temperature and filtered. The filtrate was acidified to pH 4 with the aid of hydrochloric acid (4N, in dioxane), filtered and concentrated under reduced pressure in the presence of 5-fold excess of silica. Flash chromatography (with dry loading) is performed to purify the urazole (eluent = ethyl acetate:heptane 40% to 100 % ethyl acetate)(rf = 0.22).

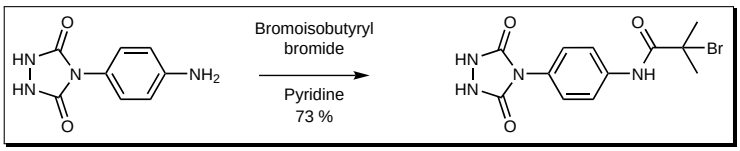
Finally, the benzyl ether was removed by dissolving the urazole (0.5 g, 1.8 mmol) in methanol (10 mL). Palladium hydroxide (5% on activated carbon) was added. Then, a balloon containing hydrogen gas was placed on the reaction, this mixture was stirred vigorously for two hours at room temperature. The solution was filtered over a syringe filter to remove the palladium hydroxide and then concentrated under reduced pressure to give the desired product (0.277 g – 82%).

Bruto formula: $C_7H_{13}N_3O_3$.

MW.: 187.20 g/mol.

^{1}H-NMR (300 MHz, DMSO−d_6): δ (ppm) = 1.10-1,40 (m, 6 H, CH_2−CH_2−CH_2−CH_2−CH_2), 3.35 (2 x t, 4 H, CH_2−CH_2−CH_2−CH_2−CH_2), 10.08 (s, 2 H, NH).

III.8.3.9 Urazole initiator for Cu(0) mediated polymerisation (56)



In a 25 mL flask, 4-aminophenyl-1,2,4-triazolidine-3,5-dione (1 g, 5.2 mmol, 1 equivalent) was dissolved in anhydrous pyridine (10 mL). This mixture was cooled to ice prior to adding α-bromoisobutyryl bromide (1.20 g, 5.2 mmol, 0.65 mL, 1 equivalent) dropwise. The mixture was slowly brought to room temperature and stirred overnight. Then water (15 mL) was added and the mixture was separated three times with ethyl acetate. The organic phase was washed once with an aqueous solution of hydrochloric acid (5 mL, 1M) and concentrated *in vacuo*. The product was dissolved in ethyl acetate, methanol and concentrated on silica *in vacuo*. Flash chromatography was conducted (eluent = (ethyl acetate:methanol:Acetic acid; 95:5:1)(petroleum ether) (2:1) rf = 0.25) yielding pure product (1.3 g – 73%).

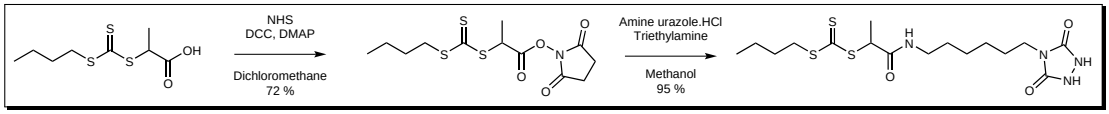
Bruto formula: $C_{12}H_{13}N_4O_3$.

MW.: 341.16 g/mol.

^{1}H-NMR (300 MHz, DMSO−d_6): δ (ppm) = 2.08 (s, 6 H, CH_3−C(Br)−CH_3), 7.45 (d, 2 H, ArH), 7.79 (d, 2 H, ArH), 10.01 (s, 1 H, Ar−NH), 10.50 (br.s, 2 H, NH).

For more experimental details see *Supporting Information file* of the publication.[62]

III.8.3.10 Urazole chain transfer agent for RAFT polymerization (59)



The chain transfer agent 2-([butylsulfanyl-carbonothioyl]sulfanyl)propanoic acid[ii] (5 g, 21 mmol, 1 equivalent), N-hydroxysuccinimide (2.9 g, 25 mmol, 1.2 equivalent) and 4-dimethyl-aminopyridine (DMAP) (257 mg, 2.1 mmol, 0.1 equivalent) were dissolved in dichloromethane (100 mL) and placed on ice and stirred. In a separate vessel N,N'-dicyclohexyl-carbodiimide (DCC) (5.19 g, 25 mmol, 1.2 equivalent) was dissolved in dichloromethane (50 mL) and this solution was added dropwise over 30 minutes to the solution containing the chain transfer agent. After one hour, the flask was removed from the ice and allowed to stir for a further 16 hours at room temperature. Subsequently, the volume of the orange solution was reduced through rotary evaporation and washed with water (2 x 200 mL) and brine (2 x 200 mL). The dichloromethane phase was collected and dried over magnesium sulphate, filtered and reduced to ≈7 mL (*in vacuo)*. Flash chromatography (eluent = ethyl acetate:n-hexanes (1 : 1 v/v) yielded pure 2-([butylsulfanyl-carbonothioyl]sulfanyl)propanoic NHS-ester(5.06 g, 72%).

Next, in a 10 mL two-neck flask, 2-([butylsulfanyl-carbonothioyl]sulfanyl)propanoic NHS-ester (128 mg, 0.38 mmol, 1 equivalent) and 4-(6-aminohexyl)-1,2,4-triazolidine-3,5-dione hydrochloride (**44**, 100 mg, 0.42 mmol, 1.1 equivalent) was dissolved in anhydrous methanol (10 mL) and placed under nitrogen atmosphere. To this, triethylamine (64 µL, 0.46 mmol, 1.2 equivalent) was added dropwise. The reaction was allowed to stir overnight at room temperature. The solvent was concentrated under reduced pressure. The resulting residue was dissolved in ethyl acetate (10 mL) and extracted with an aqueous solution of hydrochloric acid (3 x 5 mL, 1M), water (2 x 5 mL) and brine (1 x 5 mL). Flash chromatography (ethyl acetate:methanol 95:5) afforded the pure RAFT CTA (151 mg, 95%).

Bruto formula: $C_{16}H_{28}N_4O_3S_3$.

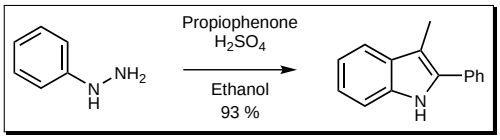
MW.: 420.61 g/mol.

MS (m/z for [MH]+): experimental: 421.10

[ii]The RAFT CTA was prepared according to literature procedures.[23]

^{1}H-NMR (300 MHz, DMSO−d_6): δ (ppm) = 0.89 (t, 3 H, CH_3), 1.21-1,68 (m, 15 H, CH_2−CH_2−CH$_3$,CH_3−CH,CH_2−CH_2−CH_2−CH_2), 3.03 (m, 2 H, NH−CH_2), 3.36 (2 x t, 4 H, CH_2−N + CH_2−S), 4.66 (q, 1 H, S−CH−CH$_3$), 8.28 (t, 1 H, NH−CH$_2$), 10.01 (br.s, 2 H, NH).

III.8.3.11 2-phenyl-3-methyl-1*H*-indole (69)



A mixture of phenylhydrazine (2.55 mL, 0.026 mol, 1 equivalent), propiophenone (3.45 mL, 0.026 mol, 1 equivalent) and concentrated sulphuric acid (11.0 mL, 1.04 mol, 2 equivalents) in ethanol (100 mL) was placed under inert atmosphere and refluxed at 85 °C. The reaction was monitored by thin layer chromatography (TLC, ethylacetate:heptane 1:9). The mixture was precipitated in a 5-fold excess of ice in water and filtrated. The residue was recrystallised in water:ethanol 1:2 to give 2,3-diphenyl-1H-indole (**69**, 5.02 g, 93%).

Bruto formula: $C_{15}H_{13}N$.

MW.: 207.28 g/mol.

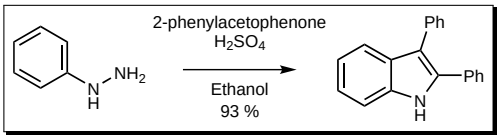
HRMS (m/z for [MH]+): calculated: 208.1121; experimental: 208.1113.

^{1}H-NMR (300 MHz, DMSO−d_6): δ (ppm) = 2.41 (s, 3 H, CH_3), 7.01 (t, 1 H, NH−C−CH=CH−CH), 7.11 (t, 1 H, NH−C−CH=CH), 7.35 (t, 2 H, ArH), 7.51 (t, 3 H, ArH), 7.67 (dd, 2 H, ArH), 11.15 (s, 1 H, NH).

^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) = 9.82 (CH$_3$), 106.74 (C), 110.99 (CH), 118.39 (CH), 118.54 (CH), 121.52 (CH), 126.94 (CH), 127.46 (CH), 128.68 (CH), 129.36 (C), 133.06 (C), 133.71 (C), 135.90 (C)

Reference: Y. Li, T. Yan, K. Junge, M. Beller, Angewandte Chemie International Edition, 2014, 53 (39), 10476–10480.

III.8.3.12 2,3-diphenyl-1*H*-indole (70)



A mixture of phenylhydrazine (10.2 mL, 0.52 mol, 1 equivalent), 2-phenylacetophenone (20.3 g, 0.52 mol, 1 equivalent) and concentrated sulphuric acid (2.75 mL, 0.052 mol, 2 equivalents) in ethanol (100 mL) was placed under inert atmosphere and refluxed at 85 °C. The reaction was monitored by thin layer chromatography (TLC, ethylacetate:heptane 1:9). The mixture was precipitated in a 5-fold excess of ice in water and filtrated. The found solids were recrystallised in water:ethanol 1:2 giving 2,3-diphenyl-1*H*-indole (25.94 g, 93%).

Bruto formula: $C_{20}H_{15}N$.

MW.: 269.35 g/mol.

^{1}H-NMR (300 MHz, DMSO−d_6): δ (ppm) = 7.04 (t, 1 H, NH−C−CH−CH−C*H*), 7.16 (t, 1 H, NH−C−CH−C*H*), 7.25-7,44 (m, 8 H, Ar*H*), 7.45-7,51 (m, 4 H, Ar*H*), 11.56 (s, 1 H, N*H*).

^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) = 111.40 (CH), 113.21 (C), 118.48 (CH), 119.61 (CH), 121.89 (CH), 125.95 (CH), 127.38 (CH), 127.89 (C), 128.08 (CH), 128.37 (CH), 128.51 (CH), 129.66 (CH), 132.39 (C), 133.99 (C), 135.216 (C), 136.02 (C)

Reference: Y. Li, T. Yan, K. Junge, M. Beller, Angewandte Chemie International Edition, 2014, 53 (39), 10476–10480.

III.8.3.13 2-formyl-3-methylphenoxy (FMP, 67)

The synthesis of 2-formyl-3-methylphenoxy (FMP) was performed according to a known literature procedure and kindly provided by the MacroArc group of Prof. Barner-Kowollik.

Bruto formula: $C_{17}H_{16}O_4$.

MW.: 284.31 g/mol.

^{1}H-NMR (400 MHz, DMSO−d_6): δ (ppm) = 2.48 (m, 3 H, CH_3−Ar), 3.88 (s, 3 H, CH_3−O), 5.35 (s, 2 H, Ar−CH_2−O), 6.90 (d, 1 H, Ar*H*), 7.13 (d, 1 H, AR*H*), 7.48 (t, 1 H, Ar*H*), 7.64 (d, 2 H, Ar*H*), 7.99 (d, 2 H, Ar*H*), 10.63 (s, 1 H, Ar−C(O)−*H*).

Reference: T. Pauloehrl, G. Delaittre, V. Winkler, A. Welle, M. Bruns, H. G. Börner, A. M. Greiner, M. Bastmeyer, C. Barner-Kowollik, Angewandte Chemie International Edition, 2012, 51, 1071–1074.

III.8.3.14 Irradiation experiment

2-Formyl-3-methylphenoxy (FMP, 5 mg, 17 µmol, 1 equivalent) and 4-Butyl-1,2,4-triazoline-3,5-dione (BuTAD, 2.7 mg, 17 µmol, 1 equivalent) were dissolved in 1 mL acetonitrile in different headspace vials (Pyrex, diameter 20 mm), which were crimped air-tight using synthetic rubber (SBR) seals with polytetrafluoroethylene (PTFE) inner liner. The solutions were deoxygenated by purging with nitrogen for 10 minutes, prior to mixing. The mixed solution was subsequently irradiated by revolving around a compact low-pressure fluorescent lamp (different lamps were used – see Figure III.19) at a distance of 40–50 mm in a custom-built photoreactor (see Figure III.22). The solvent was evaporated under vacuum after the reaction, THF (0.5 mL) was added and the solution was analyzed immediately via SEC/ESI-MS.

Bruto formula: $C_{24}H_{27}N_3O_6$.

MW.: 453.50 g/mol.

^{1}H-NMR (400 MHz, DMSO$-$d$_6$): δ (ppm) = 0.90 (t, 3 H, CH_3–CH_2–), 1.20 (m, 2 H, CH_3–CH_2–CH_2–CH_2), 1.25 (m, 2 H, CH_3–CH_2–CH_2–CH_2), 1.89 (s, 3 H, CH_3–C), 3.45 (t, 2 H, N–CH_2), 3.85 (m, 5 H, CH_3–O + Ar–CH_2–N), 5.35 (s, 2 H, Ar–CH_2–O), 6.92 (d, 1 H, ArH), 7.03 (d, 1 H, ArH), 7.48 (t, 1 H, ArH), 7.64 (d, 2 H, ArH), 7.99 (d, 2 H, ArH).

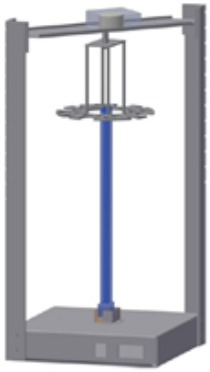


Figure III.22: Schematic representation of the custom-built photoreactor.[42]

III.9 Bibliography

[1] K. D. Collins, F. Glorius, *Accounts of Chemical Research* **2015**, *48*, 619–627.

[2] K. D. Collins, F. Glorius, *Nature Chemistry* **2013**, *5*, 597–601.

[3] R. C. Cookson, S. S. H. Gilani, I. D. R. Stevens, *Tetrahedron Letters* **1962**, *3*, 615–618.

[4] W. H. Pirkle, J. C. Stickler, *Chemical Communications (London)* **1967**, *1967*, 760–761.

[5] G. B. Butler, *Polymer Science U.S.S.R.* **1981**, *23*, 2587–2622.

[6] G. B. Butler, *Industrial and Engineering Chemistry Product Research and Development.* **1980**, *19*, 512–528.

[7] S. Radl in *1,2,4-Triazoline-3,5-Diones*, *Vol. 67*, Elsevier, San Diego, **1997**.

[8] L. H. Dao, D. Mackay, *Canadian Journal of Chemistry* **1979**, *57*, 2727–2733.

[9] K. Hoogewijs, D. Buyst, J. M. Winne, J. C. Martins, A. Madder, *Chemical Communications* **2013**, *49*, 2927–2929.

[10] R. Cookson, S. Gupte, I. Stevens, C. Watts, *Organic Syntheses* **1971**, *51*, 121.

[11] Q.-Y. Hu, M. Allan, R. Adamo, D. Quinn, H. Zhai, G. Wu, K. Clark, J. Zhou, S. Ortiz, B. Wang, E. Danieli, S. Crotti, M. Tontini, G. Brogioni, F. Berti, *Chemical Science* **2013**, *4*, 3827–3832.

[12] R. Adamo, M. Allan, F. Berti, E. Danieli, Q. Hu, *WO2013009564A1* **2012**, Tyrosine ligation process.

[13] A. Nilo, M. Allan, B. Brogioni, D. Proietti, V. Cattaneo, S. Crotti, S. Sokup, H. Zhai, I. Margarit, F. Berti, Q.-Y. Hu, R. Adamo, *Bioconjugate Chemistry* **2014**, *25*, 2105–2111.

[14] R. Adamo, Q.-Y. Hu, A. Torosantucci, S. Crotti, G. Brogioni, M. Allan, P. Chiani, C. Bromuro, D. Quinn, M. Tontini, F. Berti, *Chemical Science* **2014**, *5*, 4302–4311.

[15] R. Tsunashima, S.-i. Noro, T. Akutagawa, T. Nakamura, H. Kawakami, K. Toma, *Chemistry – A European Journal* **2008**, *14*, 8169–8176.

[16] G. Read, N. R. Richardson, *Journal of the Chemical Society Perkin Transactions 1* **1996**, 167–174.

[17] J. Burgert, R. Stadler, *Chemische Berichte* **1987**, *120*, 691–694.

[18] P. Witte, F. Beuerle, U. Hartnagel, R. Lebovitz, A. Savouchkina, S. Sali, D. Guldi, N. Chronakis, A. Hirsch, *Organic & Biomolecular Chemistry* **2007**, *5*, 3599–3613.

[19] R. Stadler, L. de Lucca Freitas, *Polymer Bulletin* **1986**, *15*, 173–179.

[20] M. Weber, R. Stadler, *Polymer* **1988**, *29*, 1071–1078.

[21] K. Matyjaszewski, N. V. Tsarevsky, *Nature Chemistry* **2009**, *1*, 276–288.

[22] C. Barner-Kowollik, *Handbook of RAFT Polymerization*, **2008**.

[23] C. J. Ferguson, R. J. Hughes, D. Nguyen, B. T. T. Pham, R. G. Gilbert, A. K. Serelis, C. H. Such, B. S. Hawkett, *Macromolecules* **2005**, *38*, 2191–2204.

[24] M. Danial, C. M. N. Tran, K. A. Jolliffe, S. Perrier, *Journal of the American Chemical Society* **2014**, *136*, 8018–8026.

[25] A. G. Leach, K. N. Houk, *Chemical Communications* **2002**, 1243–1255.

[26] C. C. Cheng, C. A. Seymour, M. A. Petti, F. D. Greene, J. F. Blount, *The Journal of Organic Chemistry* **1984**, *49*, 2910–2916.

[27] V. D. Kiselev, I. I. Shakirova, D. A. Kornilov, H. A. Kashaeva, L. N. Potapova, A. I. Konovalov, *Journal of Physical Organic Chemistry* **2013**, *26*, 47–53.

[28] V. D. Kiselev, D. A. Kornilov, L. N. Potapova, H. A. Kashaeva, A. I. Konovalov, *Russian Journal of Physical Chemistry A* **2014**, *88*, 890–892.

[29] S. Ohashi, G. B. Butler, *The Journal of Organic Chemistry* **1980**, *45*, 3472–3476.

[30] V. D. Kiselev, D. A. Kornilov, I. I. Lekomtseva, A. I. Konovalov, *International Journal of Chemical Kinetics* **2015**, *47*, 289–301.

[31] J. Casado, M. A. Lopez-Quintela, F. M. Lorenzo-Barral, *Journal of Chemical Education* **1986**, *63*, 450.

[32] M. L. Blackman, M. Royzen, J. M. Fox, *Journal of the American Chemical Society* **2008**, *130*, 13518–13519.

[33] V. O. Rodionov, S. I. Presolski, D. Díaz Díaz, V. V. Fokin, M. G. Finn, *Journal of the American Chemical Society* **2007**, *129*, 12705–12712.

[34] M. F. Debets, J. S. Prins, D. Merkx, S. S. van Berkel, F. L. van Delft, J. C. M. van Hest, F. P. J. T. Rutjes, *Organic & Biomolecular Chemistry* **2014**, *12*, 5031–5037.

[35] B. H. Northrop, R. N. Coffey, *Journal of the American Chemical Society* **2012**, *134*, 13804–13817.

[36] L. Rulíšek, P. Šebek, Z. Havlas, R. Hrabal, P. Čapek, A. Svatoš, *The Journal of Organic Chemistry* **2005**, *70*, 6295–6302.

[37] R. A. Izydore, H. E. Johnson, R. T. Horton, *The Journal of Organic Chemistry* **1985**, *50*, 4589–4595.

[38] H. Wamhoff, K. Wald, *Chemische Berichte* **1977**, *110*, 1699–1715.

[39] N. Roy, J. Lehn, *Chemistry–An Asian Journal* **2011**, *6*, 2419–2425.

[40] H. Ban, J. Gavrilyuk, C. F. Barbas, *Journal of the American Chemical Society* **2010**, *132*, 1523–1525.

[41] H. Ban, M. Nagano, J. Gavrilyuk, W. Hakamata, T. Inokuma, C. F. Barbas, *Bioconjugate Chemistry* **2013**, *24*, 520–532.

[42] T. Pauloehrl, G. Delaittre, V. Winkler, A. Welle, M. Bruns, H. G. Börner, A. M. Greiner, M. Bastmeyer, C. Barner-Kowollik, *Angewandte Chemie International Edition* **2012**, *51*, 1071–1074.

[43] C. J. Dürr, P. Lederhose, L. Hlalele, D. Abt, A. Kaiser, S. Brandau, C. Barner-Kowollik, *Macromolecules* **2013**, *46*, 5915–5923.

[44] T. Pauloehrl, A. Welle, M. Bruns, K. Linkert, H. G. Börner, M. Bastmeyer, G. Delaittre, C. Barner-Kowollik, *Angewandte Chemie International Edition* **2013**, *52*, 9714–9718.

[45] T. Pauloehrl, A. Welle, K. K. Oehlenschlaeger, C. Barner-Kowollik, *Chemical Science* **2013**, *4*, 3503–3507.

[46] Y.-C. Lai, G. B. Butler, *Journal of Macromolecular Science: Part A - Chemistry* **1985**, *22*, 1443–1461.

[47] R. C. Cookson, I. D. R. Stevens, C. T. Watts, *Chemical Communications (London)* **1966**, 744a.

[48] J. F. W. Keana, A. P. Guzikowski, D. D. Ward, C. Morat, F. L. Van Nice, *The Journal of Organic Chemistry* **1983**, *48*, 2654–2660.

[49] R. Cookson, S. Gilani, I. Stevens, *Journal of the Chemical SocietyC-Organic* **1967**, 1905.

[50] R. A. Novikov, V. A. Korolev, Y. V. Tomilov, *Russian Chemical Bulletin* **2011**, *60*, 1685–1693.

[51] Sigma-Aldrich, *4-Phenyl-1,2,4-triazoline-3,5-dione*, **2015**, `http://www.sigmaaldrich.com/catalog/product/aldrich/280992?lang=en&region=BE`.

[52] J. Lenoir, C. Bachert, J.-P. Remon, E. Adriaens, *Toxicology in Vitro* **2013**, *27*, 1954–1961.

[53] S. Mallakpour, H. Nasr-Isfahani, *Indian Journal of Chemistry* **2002**, *41B*, 169–174.

[54] J. H. Hall, O. Wong, R. Simlot, M. Miller, R. Izydore, *Anticancer Research* **1992**, *12*, 1355–1361.

[55] R. Simlot, R. A. Izydore, O. T. Wong, I. H. Hall, *Journal of Pharmaceutical Sciences* **1993**, *82*, 408–415.

[56] R. Izydore, I. H. Hall, *US4866058* **1988**, Method for control of hyperlipidemia.

[57] O. Wakabayashi, K. Matsuya, H. Ota, S. Suzuki, *DE2526358A1* **1976**, 1,2–Alkylene–4–substituted urazole herbicides.

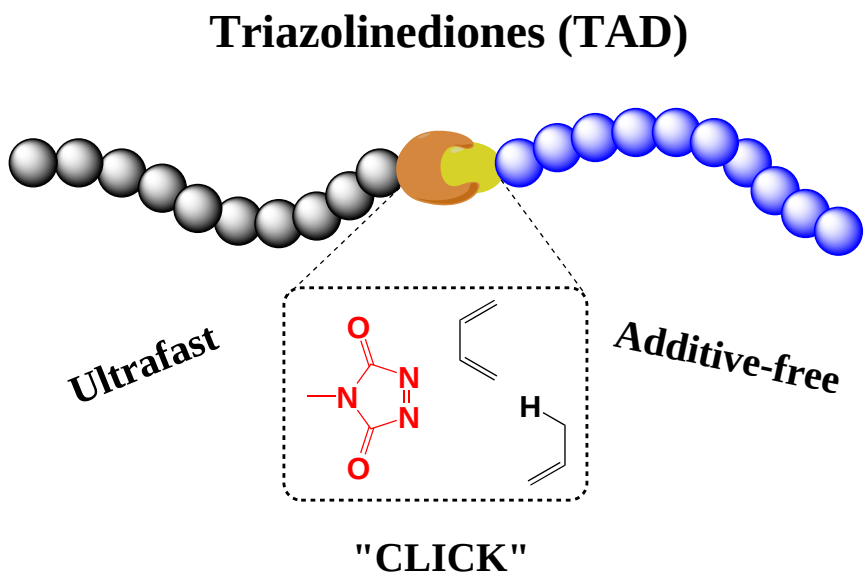
[58] O. Wakabayashi, K. Matsuya, O. Hiroki, T. Jikihara, S. Suzuki, *US4249934* **1978**, 1,2–Alkylene–4–substituted urazole herbicides.

[59] D. Ovadia, N. Peleg, P. Bracha **1977**, *US4087534*, N–haloalkylmio urazole pesticides.

[60] B. Von Bredow, H. Brechbuehler, *DE2343347* **1974**, Dichlorophenyl–2,3–diazabicyclo (2,2,1) heptane–2,3–dicarbox–imide – active against various fungi, esp, botrytis cinerea.

[61] B. Von Bredow, H. Brechbuehler, *DE2446900* **1975**, 2,3–Diaza–bicyclo (2,2,1) heptene–2,3–dicarboximides – used as pesticides, esp. insecticides.

[62] S. Billiet, K. De Bruycker, F. Driessen, H. Goossens, V. Van Speybroeck, J. M. Winne, F. E. Du Prez, *Nature Chemistry* **2014**, *6*, 815–821.



Abstract:

TAD chemistry has already been known for a long time, however this chapter will, for the first time, investigate in a detailed way the possible *click* behaviour of this chemistry, looking at both Diels-Alder and Alder *ene* reactions. First a model study on low molecular weight components is conducted. Based upon this a theoretical study is performed to rationalize the results. In a next step, this chemistry is brought to a macromolecular context (polymer-polymer conjugation). Finally network formation using TAD chemistry was examined.

References:

Billiet S., De Bruycker K., Driessen F., Goossens H., Van Speybrouck V., Winne J.M., Du Prez F.E., *Nature Chemistry* **2014**, *6*, 815-821.

Chapter IV

Development of irreversible TAD *click* reactions for polymer chemistry

IV.1 Introduction

The introduction of the concept of *click* chemistry in 2001 by Finn, Fokin and Sharpless[1,2], made a huge impact on the chemical community (as was already discussed in section II.5).[3] Although the main application was foreseen in the synthesis of biologically active molecules, other communities (e.g. the polymer community) were more prone to adapt this concept.[4] In fact, for macromolecular substrates, three additional requirements have been defined by Barner-Kowollik *et al.*, next to the original Sharpless-Finn-criteria of *click* chemistry, namely scalability (and scalable purification), equimolarity as well as a stronger emphasis on rapid reaction rates.[5]

The first additional requirement focusses on the scale of the performed reactions. When working with polymers a much larger scale, then the one that was described in the original definitions, is envisaged. This has of course major consequences to the purification of the yielded products. Simple purification methods such as distillation are not possible in a polymer context and thus the choice for purification is limited to precipitation or the removal of volatile components. However, the choice to use such a purification method depends on the physical properties of the product and not of the reaction itself. So, in most cases, this restriction leads to reactions that are performed under equimolar conditions.

This second requirement, equimolarity, is of great importance in polymer science, espe-

cially in the case of polymer–polymer conjugations. The main issue here is the difficulty to separate the desired block copolymer from its starting polymers (definitely not on a larger scale) and thus yielding the pure end product.

This equimolarity also puts requirements of the reaction kinetics of the involved reaction (cf. $\nu = k\,[A]\,[B]$). Since in the case of equimolarity, the only variable on the kinetics is the rate constant of the reaction, a strong interest grew in the polymer community towards faster reactions.

During the last years, important trends and drivers (*vide infra*) strongly influenced the implementation and method development of *click* chemistry in the field of polymer and material science. The original chemistry introduced by Sharpless[1,2] (and by Meldal in the field of synthetic polymer chemistry[6,7]) was the *copper(I)-catalyzed azide-alkyne cycloaddition* (CuAAC). The importance of CuAAC in a macromolecular context cannot be ignored as can be seen by the fact that all relevant polymerization techniques were adapted, allowing for its successful application.[3] Despite all the appealing characteristics, the use of CuAAC is not without certain disadvantages (difficulty of functionalization of the azide or alkyne, slow reaction conditions...) Maybe the most relevant drawback of the use of CuAAC is the presence of copper in the reaction media. No matter how low the concentration of the used copper is, it remains challenging to remove the residual Cu species from the reaction products, especially because the resulting triazole ring is known to complex with Cu.[8] This problem was a strong driving force to evaluate other conjugation reactions as potential *click* methodologies, as summarized in a recent perspective article by the own research group (see Figure IV.1).

The class of *metal-free click* alternatives grew rapidly when many 'old' reactions were revived again, this time in the *click* context. Examples are the well-known thiol-*ene* reactions[9,10] and the Diels-Alder[11] (DA) conjugation. Both strategies provide the possibility of combining various complementary partners - thiols and double bonds for thiol-*ene* and diene/dienophile combinations for DA. Depending on the desired end-product, a choice can be made in both reaction partner and conditions, enabling users to design synthetic strategies to their specific needs.

Besides just being a *metal-free* alternative to CuAAC, certain specific reaction conditions

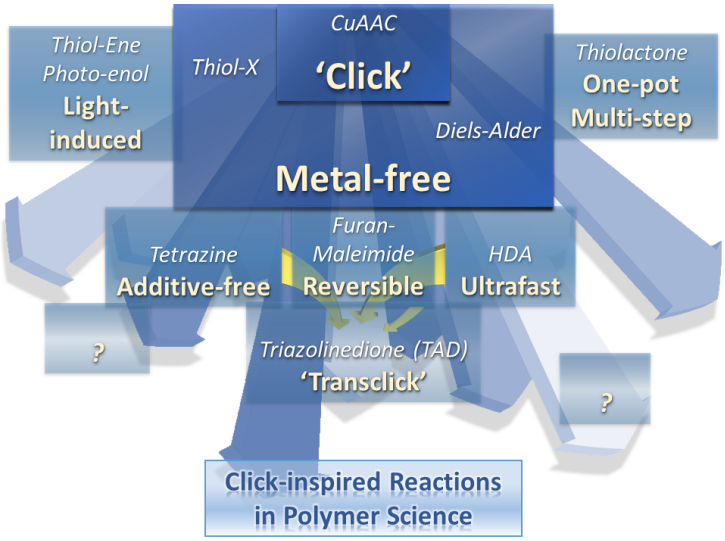


Figure IV.1: Schematic depiction of a decade of progress and developments regarding *click*(-inspired) reactions in polymer science (Reprinted with permission from [3] - copyright (2015) American Chemical Society).

could be used to introduce additional interesting characteristics. A first one is the control over a reaction in both a temporal and spatial way. This was made possible by the use of (UV) light-triggers (both in thiol-*ene* [12] and cycloadditions [13–15]). Besides this, the research community is constantly looking to simplify conjugation protocols, as more complex end products which are the result of a combination of different (and consecutive) reactions are desired. Hence, the orthogonal behaviour of *click* reactions becomes increasingly more relevant when multistep processes in a *one-pot fashion* are targeted. [16] An example of such a reaction is the use of thiolactone as latent thiol functionality, developed in our research group. [17]

In the case of cycloadditions, some additional advantages can be obtained, as reported in Figure IV.1. A first example is a characteristic that is well known for DA reactions, namely its reversibility. A popular (DA) example is the combination between a furan and a maleimide. [18] At elevated temperature, the corresponding retro-reaction occurs leading to the original starting products. This seems contradictory to the need for stable products (one of the original claims by Sharpless), however the possibilities provided by a certain extent of reversible behaviour are numerous (as can be seen by the many publications concerning dynamic covalent chemistry). [19,20] Secondly, although details about the reaction kinetics were not part of the original definition of *click* chemistry, interest grew in developing faster reactions (*vide infra*). Barner-Kowollik and co-workers came up with

a hetero-DA reaction (between a cyclopentadiene moiety and a dithioester) that showed ultrafast kinetics (full conversion in less than two hours at room temperature).[21] A last added value that cycloadditions can offer, is a built-in feedback system. For example, in the case of the (additive-free) inverse-electron-demand DA reaction between a tetrazine unit and double bonds, co-developed by our group in macromolecular context, there is a distinctive colour change during the reaction.[22]

The possibilities that *click* chemistry provides, by simply varying the conjugation strategy based on the desired characteristics, are numerous. However, tuning characteristics for a fixed reaction path is not that straightforward. In this chapter, the use of triazolinediones (TADs) as tools for such a tunable conjugation platform is introduced. As already mentioned in chapter II, TAD molecules are strong chromophores, while the corresponding adducts are colourless, thus providing a simple visual feedback mechanism. The reversible behaviour (and the tuning thereof) will be discussed in chapters VI & VII. The additive-free, ultrafast irreversible reaction pathway is the subject of this chapter. Therefore in a first step, model studies are performed to show the *click* behaviour of TAD DA & AE chemistry. This is further empowered by a theoretical rationalization, after which this is transformed to the macromolecular level for both polymer conjugation and crosslinking.

IV.2 Model study on low MW substrates

Although TAD chemistry has been known for a very long time in organic synthesis, it was never systematically studied in the context of *click* chemistry. Most likely this can be explained by the relatively lower amount of attention from the synthetic community for TAD reagents since the introduction of the *click* concept (in 2001) and its somewhat 'exotic' reputation as a highly unstable species. Whereas section II.5 gives a overview of *click-like* reactions involving triazolinediones, here the (possible) *click* behaviour of TAD chemistry will be discussed in detail. In a first step, these model studies were performed on low molecular weight compounds, to enable a complete characterization. Two pathways will be discussed in this PhD research - Diels-Alder and Alder-*ene* - and both of them will be submitted to the same model studies. For this work, the extended definition of *click* chemistry (for use in polymer science) will be used.[5] Figure I.1 gives an overview of the ideal reaction characteristics that should be fulfilled in order to be classified as *click*.

IV.2.1 TAD Diels-Alder

As a model reaction, the DA conjugation of 2,4-hexadien-1,6-diol (HDD, **71**) and 4-butyl-1,2,4-triazoline-3,5-dione (BuTAD, **31**) in DMSO-d_6 was chosen (see Figure IV.2a). HDD is a bifunctional analogue of the commercially available 2,4-hexadien-1-ol (HDEO). Both of these components will be used later in this work to introduce diene functionalities in polymers. The mentioned reaction is performed at room temperature (in presence of air) under equimolar conditions and happens immediately after mixing both compounds. As can be seen in Figure IV.2b, the reaction is quantitative (high yielding) and leads to a single reaction product (single reaction trajectory).

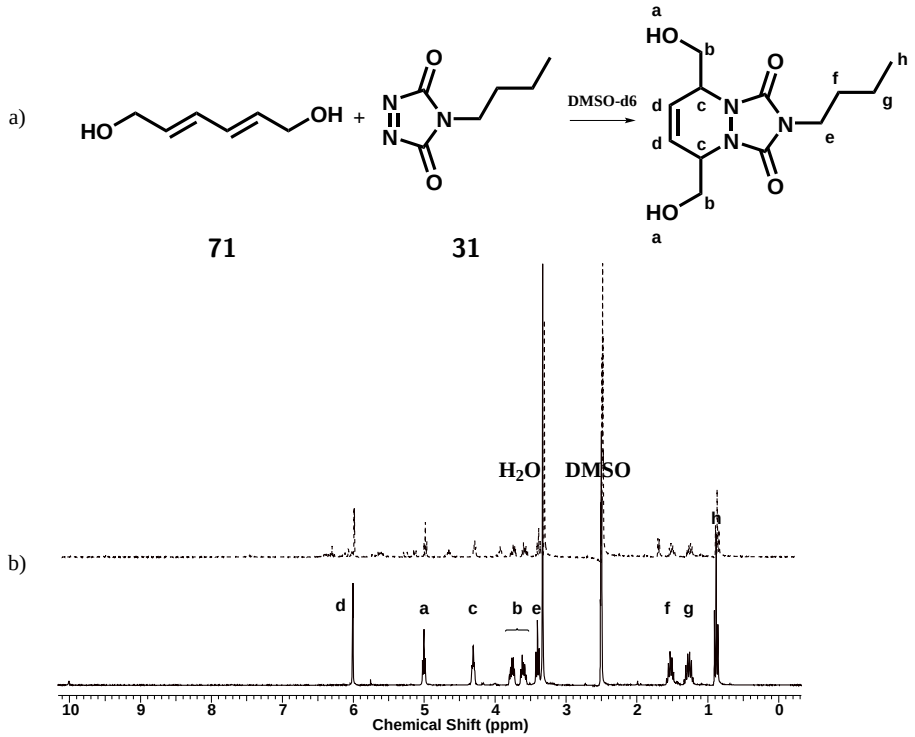


Figure IV.2: a) Model study between 4-butyl-1,2,4-triazoline-3,5-dione (BuTAD, **31**) and 2,4-hexadiene-1,6-diol (HDD, **71**) and b) ^{1}H-NMR spectrum of the Diels-Alder adduct of BuTAD and HDD (300 MHz - DMSO-d_6) - full line and mixture of the HDD-BuTAD adduct and HDEO heated to 250 °C - dashed line.

In order to assess the remaining characteristics, a quick overview of already discussed topics will be given. An important parameter (especially in polymer context) is the large-scale purification. In the case of TAD chemistry this can be rather straightforward implemented as almost all purification steps that are performed, both for the synthesis of urazoles as the oxidation to TAD, rely on precipitation, making the transfer to larger scales possible. Section III.4 showed the large rate constants for DA reactions involving

TAD moieties, in the case of the model study this is no exception (reaction is completed in less than one second). Besides this, section III.4 also showed that a certain extent of tunability was possible in kinetic behaviour. Although this is not a desired characteristic for *click* behaviour, it is an interesting advantage that cannot be overlooked. Based on the positive first results of this study, *click* behaviour was further investigated by checking the stability of the obtained product. The reason for the interest in (temperature) stability came from the original idea of this doctoral work to study dynamic systems which would require reversible connections. To check the reversible or dynamic behaviour at elevated temperature, the HDD-BuTAD adduct (obtained in DMSO-d_6) was mixed with one equivalent of HDEO and heated in a pressurized vessel up to 250 °C for a prolonged period (more than one hour) - see Figure IV.2, dashed line. The TAD–HDD adduct proved to be thermally stable as it was fully recovered from these reactions and no trace of the possibly TAD–HDEO cycloadducts or other side products were observed. The orthogonality was already discussed in section III.2, where it was shown that the reaction is very chemoselective. Although amines may be a potential threat to the efficiency of the reaction, all other functional groups do not hinder the process at all. And last (but not least), the modular behaviour has to be taken into account, which can be closely related to the *wide-in-scope* character. The large number of (commercially available) dienes that can be used in combination with TAD readily makes this a modular reaction. The fact that TAD can be used in both material science[23] and biomedical applications[24] shows even more the potential of this 'exotic' chemistry.

It has to be mentioned that not all *click* applications discussed earlier (section II.5) use TAD DA chemistry, but it is expected that this will increase when the chemistry is more known in the chemical community. It can be seen that TAD DA chemistry possesses all desired characteristics to get the label of being a versatile *click* reaction and even adds some additional benefits such as the visual feedback and kinetic tunability.

IV.2.2 TAD Alder-*ene*

The same investigation has been performed on an Alder-*ene* (AE) TAD reaction. The model reaction is in this case the equimolar reaction between BuTAD (**31**) and 2,3-dimethyl-2-butene (DMB, **72**) – see Figure IV.3a. The *ene* was chosen for its interesting kinetic behaviour (see section III.4) and its commercially availability. The quantitative (and clean) adduct formation can be seen in the ^{1}H NMR spectrum (see Figure IV.3b). Again, the reaction can be assessed as a function of its *click* behaviour. Similar as with the DA reaction, it is evident that the TAD AE reaction scores highly on equimolarity, purification and yields. Chapter III.4 showed that AE reactions are slower than DA conjugations, however there is a greater range of rate constants available, making this an even more versatile reaction pathway.

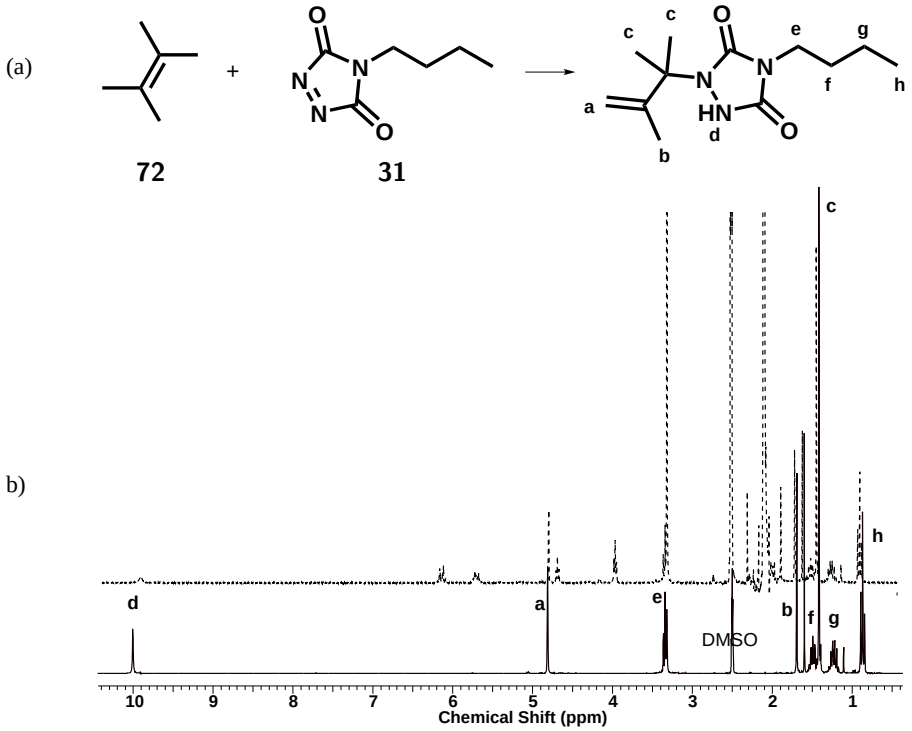


Figure IV.3: a) Model study between 4-butyl-1,2,4-triazoline-3,5-dione (BuTAD, **31**) and 2,3-dimethyl-2-butene (DMB, **72**) and b) ^{1}H-NMR spectrum of the Alder-ene adduct of BuTAD and DMB (300 MHz - DMSO-d_6) - full line and mixture of the DMB-BuTAD adduct and HDEO heated to 250 °C - dashed line.

With regard to chemoselectivity (section III.2.2), the same can be concluded as for the DA reaction, except that this time, amines and (logically) dienes have to be avoided completely. To assess the stability of the formed adduct, the same experiment was performed as for the DA reaction (addition of HDEO and heating in a pressurized vessel to 250 °C).

Similar results were obtained, leading to the conclusion that the adduct from the AE TAD reaction is stable to higher temperatures. Also concerning the modular behaviour, this reaction is very favourable. The availability of *enes* is much higher than that of dienes, especially in natural components, which will be the topic of a separate chapter in this doctoral work (see chapter V). Additionally, the stability of *enes* also compares favourably to that of most dienes, as *enes* are much less prone to typical diene side reaction such as reactions with electrophiles and/or self-polymerisation.

Based on these model studies, the *click* behaviour of TAD chemistry (in both DA and AE) becomes apparent. In order to strengthen our understanding of these chemistries, a collaboration was started with the *Center for Molecular Modeling* at Ghent University to support this statement with a theoretical rationalization for both reaction pathways.

IV.3 Computational study: theoretical rationalization

In light of the promising results described above, the mechanisms of the TAD based *click* reactions were investigated by means of *density functional theory* (DFT) calculations. Sharpless and co-workers noted that *click*-reactions should be spring-loaded, meaning that they should exhibit a large thermodynamic driving force (>84 kJ/mol) to favour a reaction with a single reaction product.[1]

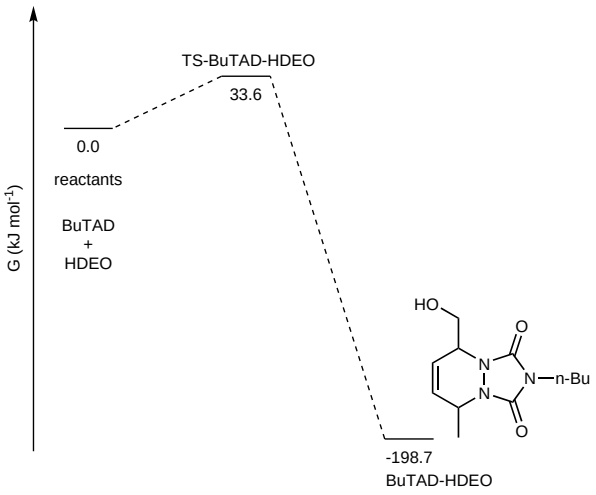


Figure IV.4: Free energy profile (kJ/mol) for the TAD-HDEO reaction (PCM ($\epsilon = 8.93$) M06-2X/6-31+G(d,p).

In the case of the TAD DA reaction between BuTAD and HDEO the reaction proceeds, as expected, via a concerted mechanism ($\Delta G^{\neq}$ = 33.6 kJ/mol). The calculated relative product stabilities and the associated barriers for the reverse reaction, regenerating BuTAD and HDEO, are in good agreement with the experimental observations that the reaction is irreversible (barriers for reverse reactions exceed 200 kJ/mol) and thus certainly fit this particular *click* reaction criterion (see Figure IV.4).

The same calculations were performed for the TAD AE reaction. Initially, TAD-ene reactions were believed to proceed via a concerted mechanism. However, it has been previously demonstrated that the reactions follow a stepwise route via an aziridinium ylide intermediate (AI) or an open zwitterionic intermediate (OZ).[23] In order to get a good model study for all *enes*, the reaction between BuTAD and a simple olefin (pent-3-en-1-ol) was chosen. Figure IV.5 shows that the reaction proceeds via an aziridinium ylide intermediate ($\Delta G^{\neq}$ = 68.0 kJ/mol). Again, the calculated relative product stabilities and the barriers for the retro reaction show that the TAD AE can be described as an irreversible *click* reaction.

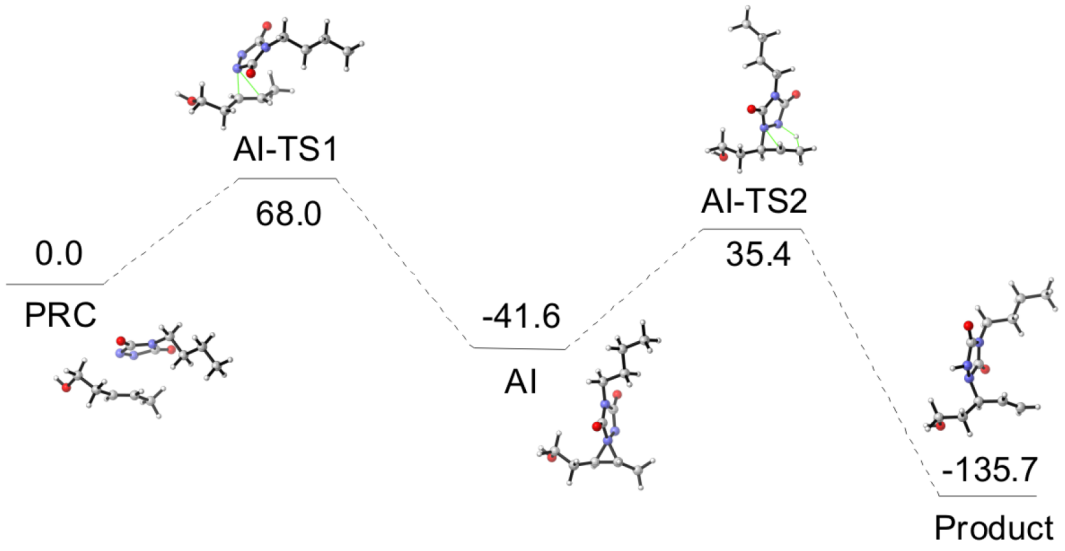


Figure IV.5: Free energy profile (kJ/mol) for the BuTAD-pent-3-en-1-ol reaction (PCM (ϵ = 8.93) M06-2X/6-31+G(d,p), PRC stands for pre-reactive complex and AI for aziridinium ylide intermediate).

In general, it can be concluded that the calculated (relative) product stabilities confirm the irreversible 'spring loaded' *click* behaviour of both TAD DA and AE reactions.

IV.4 Macromolecular functionalization and polymer conjugation using TAD-based *click* chemistry

After having established the *click* character of TAD chemistry, the next step was to bring this to a macromolecular level. To achieve this, a polymer-polymer conjugation (equimolarity is a key step here) was explored. As an intitial test of *TAD-click* based polymer conjugations, first the coupling between a polymer and a low molecular weight component was investigated. This will provide a more easy characterization before going to the more challenging polymer-polymer conjugations. For this purpose, suitable end-group functionalized linear polymers were synthesized and reacted with a low-molecular-weight TAD compound (BuTAD). Using a procedure developed by Barner-Kowollik and coworkers[25], a polyisobornylacrylate (PIBA) with a terminal cyclopentadiene (Cp) moiety, one of the most reactive dienes in Diels–Alder reactions (see section III.4), was prepared. This diene-functionalized polymer, PIBA–Cp (number average molecular weight (M_n) = 3000 g/mol and dispersity ($Đ$) = 1.19), was dissolved in THF and mixed in a 1:1 molar ratio with BuTAD (see Figure IV.6).

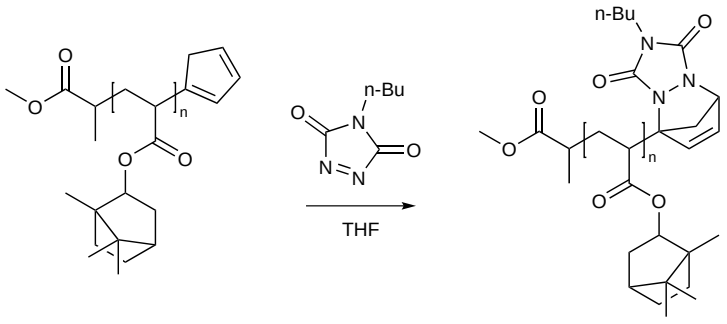


Figure IV.6: Reaction scheme of *click* reaction between polyisobornylacrylate with cyclopentadiene end group (PiBA-Cp) and BuTAD.

The reaction was completed within seconds, as judged by the disappearance of the distinctive colour of the BuTAD reagent. The resulting polymer was characterized by ^{1}H-NMR spectroscopy (see Figure IV.7), size-exclusion chromatography (SEC) (M_n = 3700 g/mol, $Đ$ = 1.20) and matrix-assisted laser desorption ionization–time-of-flight mass spectroscopy (MALDI). These analyses showed a complete conversion into the BuTAD Diels–Alder adduct with no sign of side reactions.

The same results were obtained when a polystyrene backbone was used (PS–Cp, M_n =

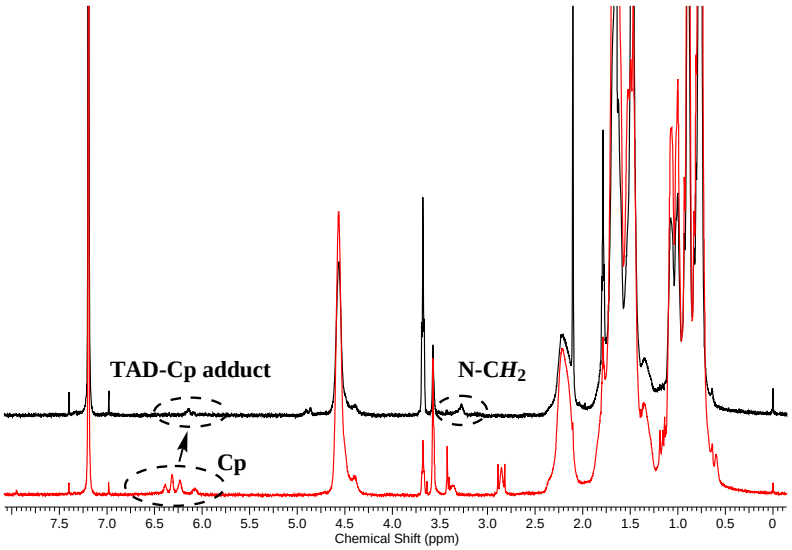


Figure IV.7: ^{1}H NMR spectrum of PiBA-Cp (red) and PiBA-Cp + BuTAD (black) in $CDCl_3$ with assignment of relevant peaks.

4000 g/mol, $Đ = 1.1$). Furthermore, instead of the Cp group, an HDEO-derived moiety was introduced as an end group onto the same polymer backbones and the reactions with BuTAD were found to proceed with the same efficiency.

Following the demonstration of efficient *clicking* of a small TAD molecule on a polymer substrate, the next step was to investigate the block copolymer formation through an (irreversible) TAD-based *click* reaction between two polymers. To obtain a TAD-functionalized polymer, a urazole-derived initiator was prepared (see III.8.3.9) for the Cu-mediated controlled radical polymerization of butylacrylate (collaboration with Frank Driessen). This urazole-end-capped poly(butylacrylate) (PBA–urazole, $M_n = 20000$ g/mol, $Đ = 1.38$) was then oxidized (in DCM with DABCO-Br for three hours) to give the corresponding polymer-bound TAD reagent (PBA–TAD). As expected, this polymer showed the same distinctive colour as the other TAD components. After dissolving PBA–TAD and PS–Cp in THF, the distinctive red colour disappeared in a matter of seconds, which indicated an ultrafast, quantitative polymer–polymer conjugation reaction in an additive-free way under ambient conditions. The obtained reaction mixture was first analysed by LCx-SEC (see Figure IV.8). In each elugram, the y axis shows a separation on the first (LC) dimension, whereas the x axis depicts the separation of collected fractions according to the hydrodynamic volume (SEC) in the second dimension. After linking the homopolymers, a new signal is visible, which represents the newly formed block copolymer, characterized by an altered behaviour in both hydrophobicity

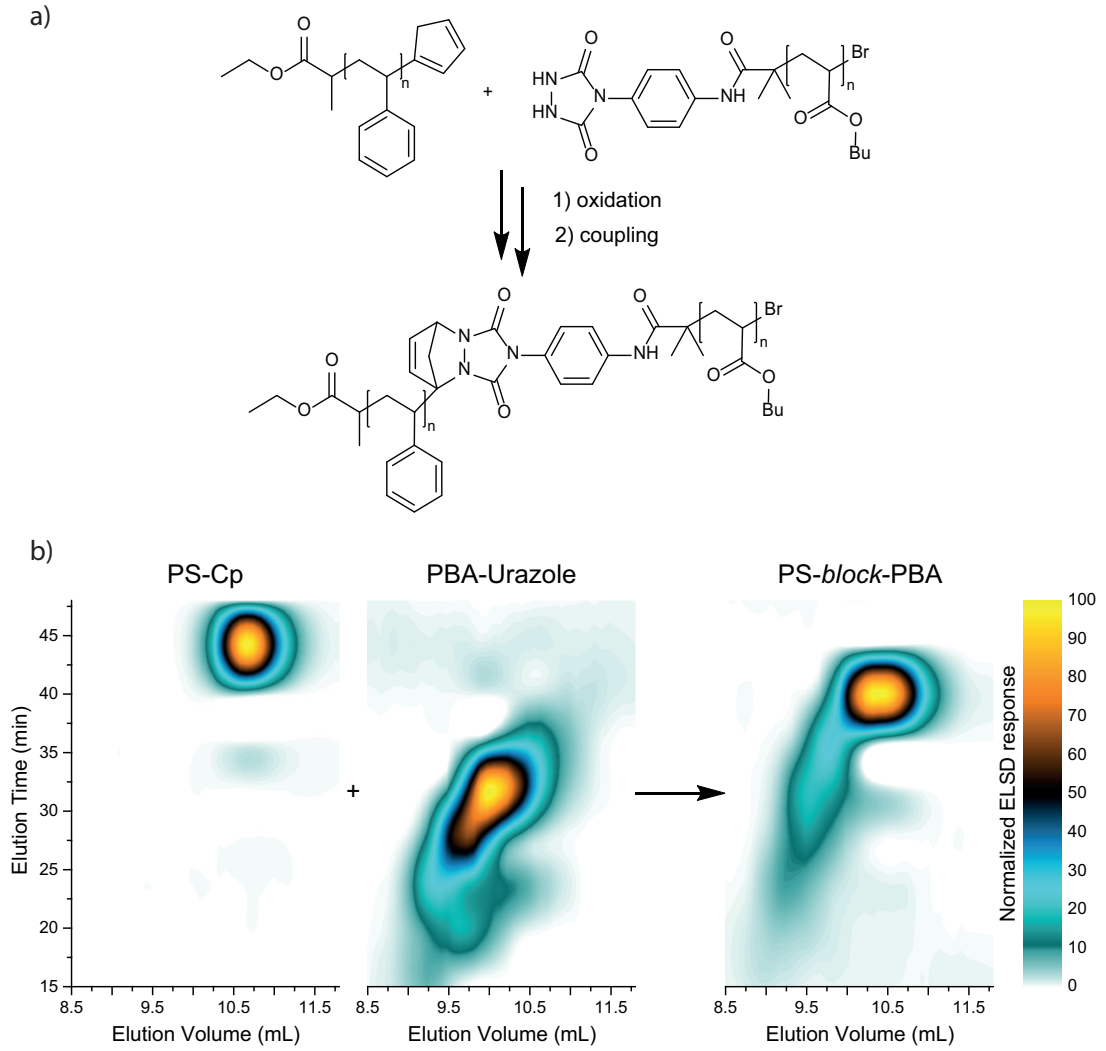


Figure IV.8: a) Oxidation of polybutylacrylate-urazole (PBA–urazole) to yield PBA-TAD, followed by coupling with polystyrene-Cp (PS–Cp) and b) LCx-SEC analysis of separate polymers (PS–Cp and PBA–urazole) and the obtained block copolymer.

and hydrodynamic volume. The small tailing in the corresponding elugram is ascribed to a small deviation from equimolarity because of the dispersity of both polymers. In a second step the coupling was confirmed by ^{1}H-NMR analysis.

IV.5 Synthesis of polymer networks using TAD-based crosslinkers

As a final demonstration of the possibilities of TAD-based *click* reactions in macromolecular systems, the ultra-fast synthesis of polymer networks was investigated. For this, we have chosen for the synthesis of polyurethane networks. In this context, two different methods were tested. In the first one, a linear polyurethane was prepared - from

a diene-containing diol (2,4-hexadiene-1,6-diol (HDD) - **71**) in combination with hexamethylene diisocyanate (HDI) and polypropylene oxide (PPO) - that was subsequently crosslinked with the bifunctional TAD molecule 4,4'-(4,4'-diphenylmethylene)-bis-(1,2,4-triazoline-3,5-dione) (MDI-TAD - **9**). The crosslinking process occurred instantaneously as indicated by the disappearance of the red colour. However, due to the limited handling time (crosslinking occurs with seconds), it proved to be very challenging to obtain large homogeneous samples. Therefore, in the second method, MDI-TAD was first reacted with an excess of HDD in order to form the crosslinks prior to polymerization. Again, the TAD reaction proceeded very fast and the resulting products could again be mixed with HDI and PPO (in the exact same ratio) to form an identical polyurethane network (see Figure IV.9 and IV.3.7.11). In this way (larger) homogeneous samples could be obtained.

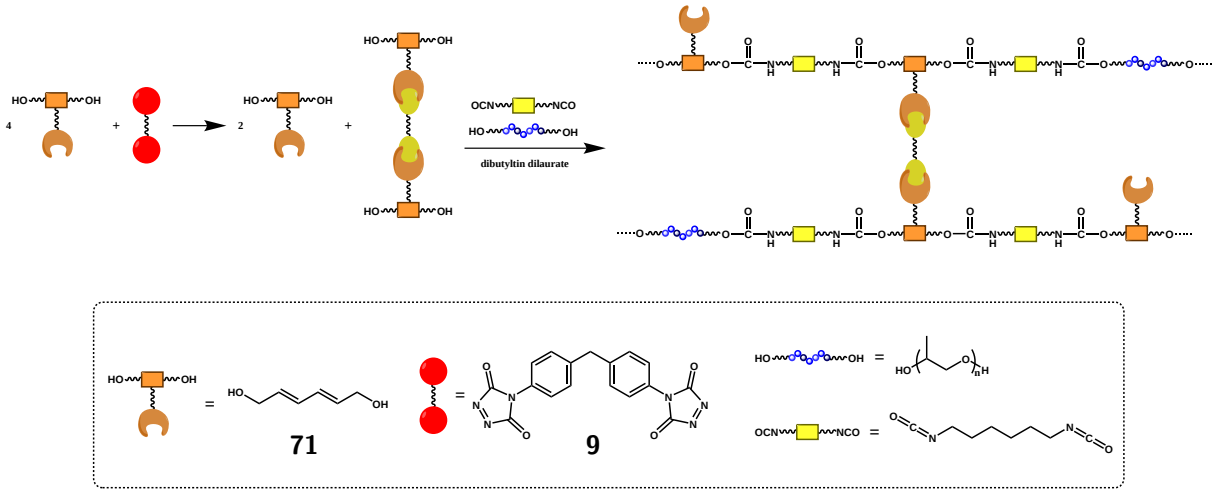


Figure IV.9: Synthesis of polyurethane networks with the crosslinking based on TAD DA *click* chemistry.

Once these materials were prepared, the network properties were studied. By means of TGA and DSC analysis, it was determined that the obtained rubbers ($T_g = -50\,°C$) were stable up to high temperatures ($T_{deg} = 300\,°C$). Following this, the networks were subjected to harsh conditions (refluxing high boiling solvents) to assess the thermoset behaviour. Materials (synthesized via both methods) showed completely insoluble behaviour in refluxing DMF, even when heated at 200 °C in a pressurized vessel and in the presence of a competing diene (HDEO).

As an excess of diene groups (2:1) was incorporated into the polymer network, possible transfer reactions could also be determined via simple qualitative testing. For this the

material was damaged (by a razor blade) and subjected to higher temperatures to provoke a thermal healing process. Additionally the material was broken into smaller pieces and put into a mould under pressure for 30 minutes at 120 °C. After both tests, the original samples were recovered, which led to the conclusion that indeed irreversible bonds were obtained via the DA TAD reaction.

IV.6 Conclusions and perspectives

Triazolinediones are highly reactive moieties that have been known for a very long time in the field of organic synthesis. However, since the introduction of the *click* chemistry concept, these molecules were not assessed yet for their ability to be used in such a process. This chapter tested both Diels-Alder and Alder-*ene* reactions, involving TAD molecules, for their *click-like* behaviour.

Firstly, model studies were performed and fully characterised to assess the *click* properties of both reaction pathways. This was done by checking all desired specifications that are necessary to be labelled as a *click* reaction. To assure relevance for polymer chemistry application, the 'expanded' criteria for *click* reactions - introduced by Barner-Kowollik *et al.*[5] - was used. The positive outcome of these initial experiments was further reinforced by theoretical calculations. Once the clear *click* behaviour of TAD chemistry was established, the step was made towards macromolecular functionalization. For this, the (ultrafast) formation of block copolymers being was studied. The final research line focussed on the synthesis of networks.

This chapter investigated the *click* behaviour of TAD chemistry. The successful experiments resulted in some applications that were also briefly looked upon. However, a vast number of possibilities remains available for future research. In order to get the chemistry more known to the general chemical community, it is necessary to showcase this versatile coupling strategy in as many research domains as possible, an effort that is ongoing and in the meantime partially accomplished within the PCR group.[26–28]

IV.7 Experimental section

IV.7.1 Materials

Acetic acid (glacial), aluminiumoxide (activated, basic), bromine (reagent grade), α-bromoisobutyryl bromide (98%), butyl acrylate (>99%), celite®, citric acid (>99.5%), copper(I)bromide (99%), copper(I)iodide (98%), Cu(0) was used as pellets (>99.999%), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 98%), N,N-diisopropylethylamine (DiPEA, 99.5%), tris[2-(dimethylamino)-ethyl]amine (Me_6TREN, 97%), 2,3-dimethyl-2-butene (99%), ethyl 2-bromopropionate (99%), *trans*, *trans*-2,4-hexadien-1-ol (>97%), isobornyl acrylate (technical grade), lithium aluminium hydride (LAH, powder, 95%), methyl 2-bromopropionate (98%), 4,4'-methylenebis(phenyl isocyanate) (98%), 4-nitrophenyl isocyanate (97%), palladium on carbon (5%), N,N,N',N",N"-pentamethyldiethylenetriamine (PMDETA, 99%), potassium hydroxide (reagent grade, 90%, flakes), propargyl alcohol (99%), sodium iodide (>99.5%), succinic anhydride (>99%), N,N,N',N'-tetramethylethylenediamine (TMEDA, 99%) and tributylphosphine (97%) were purchased from Sigma-Aldrich; butyl isocyanate (>98%), dibutyltin dilaurate (>95%) and nickelocene ($NiCp_2$, >98%) from TCI; hydrochloric acid (36%) from Chem-Lab. Ammonium chloride (99%), potassium carbonate (99%) and sodium bicarbonate (99.5%) from Roth. Hexamethylene diisocyanate (>98%) from Fluka, magnesium sulfate (anhydrous) from Boom. Ethyl carbazate (97%), polypropylene oxide (Mn $\approx$ 2000 g/mol) and sodium hydroxide (97%) from Acros Organics. All solvents (Sigma-Aldrich) and products were used without any pre-treatment or purification.

Styrene (Sigma-Aldrich, $\geq$99%) was passed over a short column of Al_2O_3 prior to use. N,N,N',N",N"-Pentamethyldiethylenetriamine (PMDETA, Sigma-Aldrich, 99%) was distilled (85 −86 °C, 16 mbar) and stored at 4 °C. Cu(I)Br (Sigma-Aldrich, 98%) was purified by stirring with acetic acid, then by filtering and washing with ethanol and diethylether, and finally by drying in a vacuum oven at 70 °C.

IV.7.2 Characterization

Nuclear Magnetic Resonance (NMR) ^{1}H-spectra were recorded with a Bruker Avance 300 (300 MHz) FT-NMR spectrometer in $CDCl_3$ (Eurisotop) or DMSO-d_6 solution at

room temperature. Chemical shifts are presented in parts per million (δ) relative to $CHCl_3$ (7.26 ppm for ^{1}H-NMR) and DMSO (2.50 ppm for ^{1}H-NMR) as an internal standard. The resonance multiplicities are descried as [br. (broad)] s (singlet), d (doublet), t (triplet), q (quadruplet), quin (quintuplet), sext (sextuplet) or m (multiplet).

Differential Scanning Calorimetry (DSC) thermograms were recorded using a TA Instruments Q2000 DSC with autosampler option and Refrigerated Cooling System (RCS). Nitrogen gas was used as purge gas. The samples were studied in TAi Tzero Hermetic aluminium sample pans and at a scan rate of 10 K min^{-1}.

Thermogravimetric analysis (TGA) was performed using a Mettler-Toledo TGA/SDTA 851e equipment. Samples (5 to 10 mg) were heated in a nitrogen atmosphere with a heating rate of 10 K min^{-1} going from 25 °C to 600 °C. For the analysis of the thermograms, the STARe software of Mettler-Toledo was used.

LCx–SEC analysis. For two-dimensional LC, sample fractions from the first dimension were transferred to the second-dimension column via an electronically controlled eight-port valve system (VICI Valco instruments), equipped with two 200 µL sample loops. The second dimension consisted of an Agilent Infinity 1260 isocratic pump and a PSS SDV LIN M 200 µm column. Detection in the second dimension was accomplished by using an evaporative light-scattering detector (ELSD). Nitrogen was used as the carrier gas in the ELSD at a flow rate of 2.5 L/min. Spray chamber, drift tube and optical cell temperatures were set at 30 °C, 80 °C and 70 °C, respectively. The flow rates used in the first and second dimensions were 0.05 mL/min and 5 mL/min, respectively. Sample concentrations were between 0.25 and 2.0 mg/ml. THF was used as the solvent for the second dimension analysis. Data were recorded using PSS WinGPC Unichrom software.

Computational methodology All measurements were performed by Dr. Hannelore Goossens of the *Centre of Molecular Modeling* (CMM) of *Ghent University*. To start with, the B3LYP/6-31+G(d,p) level of theory was used for geometry optimizations. A thorough conformational analysis was performed on all reactants, transition states, intermediates and products to identify the most plausible conformers. The stationary points were characterized as minima (ground states) or first order saddle points (transition states) by normal modes analysis. Intrinsic reaction coordinate (IRC) calculations were used to

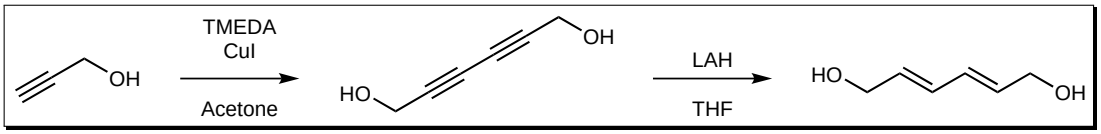
verify the corresponding reactant and product complexes. The B3LYP functional has been proven to produce good geometries but is less accurate for energy calculations. Therefore energies were refined with the M06-2X functional, which is able to account for dispersion effects, and a 6-31++G(d,p) basis set. Since the studied reactions take place in dichloromethane, which cannot form hydrogen bonds with the reactive substrate, the solvent environment was taken into account by means of a continuum model. From preliminary calculations, where a polarizable continuum model (PCM) was only taken into account for energy refinement, it was found to be necessary to take PCM into account for geometry optimizations. Thermal free-energy and enthalpy corrections were taken from B3LYP/6-31+G(d,p) optimizations at 1 atm and 298,15 K. Next, the M06-2X/6-31+G(d,p) level of theory, in combination with PCM, was used for geometry optimizations of the most plausible reaction paths, since dispersion effects were assumed to play an important role in the reactions. All computation were carried out with the Gaussian 09 program package.

IV.7.3 Synthesis

The synthesis of the following compounds can be found in previous chapter:

- 4-Butyl-1,2,4-triazoline-3,5-dione (BuTAD, **31**) - see III.8.3.1
- 1,4-diazabicyclo[2.2.2]octane bromide complex(DABCO-Br) - see III.8.3.2
- Urazole initiator for Cu(0) mediated polymerization (**56**) - see III.8.3.9

IV.7.3.1 2,4-hexadiene-1,6-diol (HDD, 71)



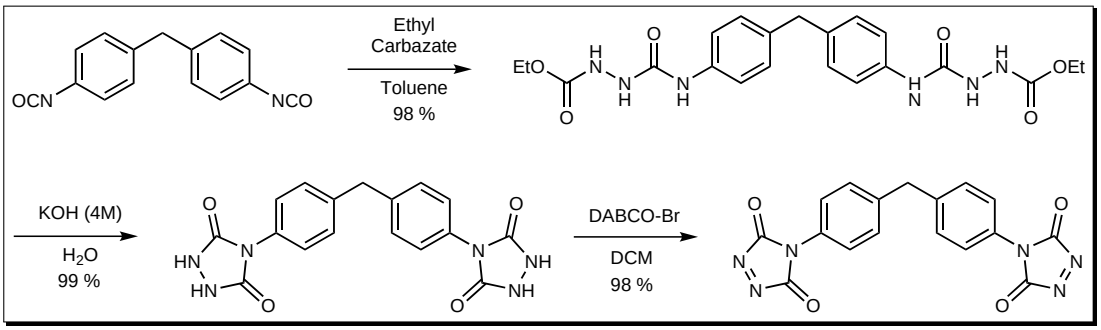
In a 50 mL one-neck flask, cupper(I)iodide (0.227 g, 1.19 mmol, 0.05 equivalent) and tetramethylenediamine (TMEDA, 0.357 mL, 2.38 mmol, 0.1 equivalent) was dissolved in acetone (20 mL). The reaction mixture was vigorously stirred open to the air and propargyl alcohol (1.00 g, 23.8 mmol, 1 equivalent) in acetone (10 mL) was added slowly. Then, the solution was stirred at room temperature for 2.5 h (open to the air - the mixture turned dark blue/black). After concentrating under reduced pressure, the residue was

dissolved in ethyl acetate and extracted with a saturated ammonium chloride solution until the organic phase was not blue anymore. The combined organic phases were washed with water (once) and brine (twice) before drying over anhydrous magnesium sulphate and concentrating under reduced pressure to obtain 2,4-hexadyne-1,6-diol.

In the next, a solution of lithium aluminium hydride (LAH, 0.72 g, 6.54 mmol) in THF (15 mL) was prepared in a 50 mL one-neck flask. To this mixture 2,4-hexadyne-1,6-diol in THF (15 mL) was added dropwise. The mixture was refluxed for four hours and subsequently cooled to 0 °C. After cooling down, water (0.75 mL) was carefully added, followed by a sodium hydroxide solution (0.75 mL, 15 %) and water (0.75 mL). This was stirred at room temperature for two hours after which the mixture was filtrated. The filtrate was concentrated under reduced pressure to obtain pure 2,4-hexadiene-1,6-diol (2.00 g - 74 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) = 4.03 (t, 4 H, 2 x HO−CH_2), 4.77 (t, 2 H, HO−CH_2), 5.80 (m, 2 H, CH−CH−CH−CH), 6.24 (m, 2 H, CH−CH−CH−CH).

IV.7.3.2 4,4'-(4,4'-diphenylmethylene)-bis-(1,2,4-triazoline-3,5-dione) (MDI-TAD - 9)



A mixture of ethyl carbazate (40 g, 0.384 mol, 2 equivalents) and toluene (300 mL) was placed in a three neck flask (1 L) and cooled in an ice bath. The flask was equipped with an addition funnel, containing 4,4'-methylenebis phenyl isocyanate (48 g, 0.192 mol, 1 equivalent) dissolved in toluene (200 mL), a mechanical stirrer and a bulb condenser. The mixture was put under inert atmosphere and the isocyanate was added slowly under vigorous stirring. After addition, the mixture was stirred at room temperature for two hours, followed by two hours at 90 °C. After cooling down the reaction to room temperature,

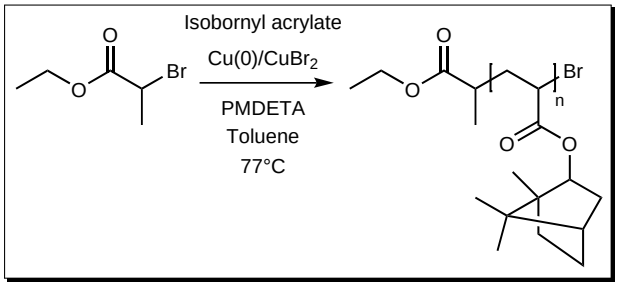
4,4'-(4,4'-diphenylmethylene)-bis-(carbethoxysemicarbazide) was filtered off and washed with toluene (98 %).

Then in a 1 L flask, the obtained semi-carbazide (86.2 g, 0.188 mol) was dissolved in an aqueous potassium hydroxide solution (330 mL, 4 M) under inert atmosphere. This mixture was refluxed for 1.5 h (100 °C), warm filtered, cooled to room temperature and acidified until pH 1 by adding of hydrochloric acid (37%). This mixture was cooled to room temperature to yield a white powder that was filtered off (99 %).

In a last step, a mixture of the just obtained 4,4'-(4,4'-diphenylmethylene)-bis-(urazole) (2 g, 5.64 mmol, 1 equivalent), DABCO-Br (5 g, 3.18 mmol, 0.29 equivalent) and dichloromethane (30 mL) was put in a flask (100 mL) under inert atmosphere and stirred for five hours at room temperature. The reaction mixture was filtered off, the residue washed with dichloromethane (2 x 30 mL) and the filtrate was concentrated *in vacuo* to obtain 4,4'-(4,4'-diphenylmethylene)-bisTAD as pink crystals (98 %). [i]

^{1}H-NMR (300 MHz, DMSO$-$d$_6$): δ (ppm) = 4.11 (s, 2 H, Ar$-$CH_2-Ar), 7.38 (d, 4 H, Ar$-H$), 7.48 (d, 4 H, Ar$-H$).

IV.7.3.3 Polyisobornylacrylate-bromide (PiBA-Br)



Isobornyl acrylate (14 mL, 66.3 mmol, 100 equivalents), ethyl acetate (4.7 mL, 25 vol%) and pentamethyldiethylenetriamine (PMDETA, 103.8 μL, 0.5 mmol, 0.75 equivalent) were weighed into a flask and degassed for one hour with a continuous nitrogen sparge. Cu(I)Br (47.53 mg, 0.33 mmol, 0.5 equivalent) was added under nitrogen atmosphere and the reaction mixture was stirred until a homogeneous solution was obtained. Afterwards, methyl 2-bromopropionate (151 µL, 6.63 µmol, 1 equivalent), which was degassed separately, was

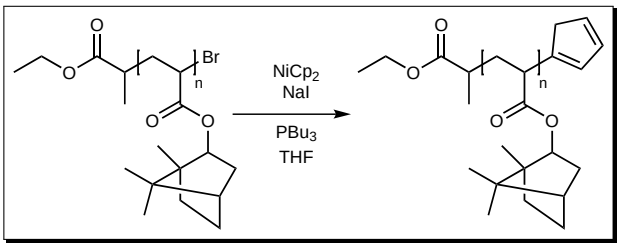
[i] The temperature of the cooling bath should not exceed 50 °C due to the volatility of the obtained compound.

added and the reaction mixture was placed in an oil bath at 77 °C. After one hour, the reaction was stopped (18 % conversion) by cooling in liquid nitrogen under air atmosphere and precipitation in cold methanol (200 mL). The precipitate was filtered off and dissolved in tetrahydrofuren (THF, 100 mL). The copper catalyst was removed by passing the reaction mixture over a column of Al_2O_3. After evaporating the excess solvent until a volume of 20 mL, the polymer was precipitated in cold methanol (200 mL). The polymer was filtered, washed with methanol and again dissolved in THF (20 mL). Finally, the polymer was precipitated in cold methanol (200 mL) and dried overnight in a vacuum oven at 40 °C.

$\boldsymbol{M_n}$ **(SEC)**: 2900 g mol^{-1}, $\boldsymbol{M_w}$ **(SEC)**: 3600 g mol^{-1}, $\boldsymbol{Đ}$ **(SEC)**: 1.23

Reference: Dervaux, B.; Van Camp, W.; Van Renterghem, L.; Du Prez, F. E., Journal of Polymer Science: Part A Polymer Chemistry **2008**, 46, (5), 1649-1661.

IV.7.3.4 Polyisobornylacrylate-cyclopentadiene (PiBA-Cp)

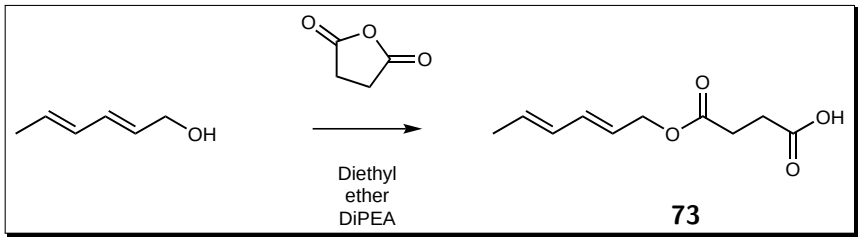


Bromide terminated PiBA (0.18 mmol) was mixed with tributylphosphine (89 µL, 0.36 mmol) and sodium iodide (0.162 g, 1.08 mmol) and dissolved in dry tetrahydrofuran (THF, 2.0 mL). This solution was placed under nitrogen atmosphere. Separately, a stock solution of $NiCp_2$ in dry THF (0.18 mol/L) was prepared under a nitrogen atmosphere. The $NiCp_2$ solution (2.0 mL) was added to the polymer solution and this mixture was stirred overnight at room temperature. After the reaction went to completion, the solution was filtrated over a short column of basic alumina, to remove the precipitated nickel(II)bromide. Then the polymer was precipitated in cold methanol, filtrated and washed thoroughly with methanol. The resulting powder was then dissolved in chloroform and washed three times with distilled water. To obtain the PiBA-Cp, the polymer was again precipitated in cold methanol, filtrated, washed thoroughly with methanol and dried in a vacuum oven overnight at 40 °C.

$\boldsymbol{M_n}$ **(SEC)**: 3700 g mol^{-1}, $\boldsymbol{M_w}$ **(SEC)**: 4500 g mol^{-1}, $\boldsymbol{Đ}$ **(SEC)**: 1.21

Reference: Inglis A.J.; Paulöhrl T., Barner-Kowollik C. Macromolecules **2010**, 43, 33-36.

IV.7.3.5 Hexadiene-1-ol derivative (73)



A mixture of *trans,trans*-2,4-hexadien-ol (2.55 g, 26 mmol, 1 equivalent), succinic anhydride (3.0 g, 30 mmol, 1.15 equivalent) and N,N-diisopropylethylamine (DiPEA, 3.4 g, 26 mmol, 1 equivalent) in diethyl ether (10 mL) was stirred for two days at ambient temperature. The solvent was removed under reduced pressure and the residue dissolved in DCM and washed with aqueous solution of 5% w/w citric acid. The organic layer was dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure to give the desired product (**73**, 14.3 g, 80%).

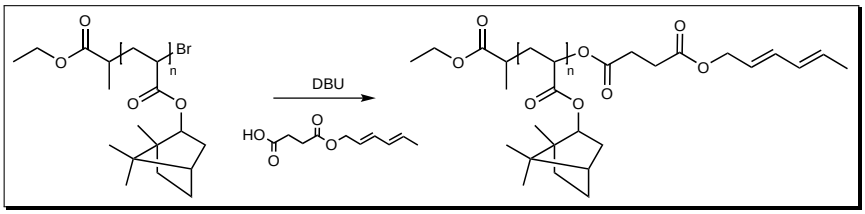
Bruto formula: $C_{21}H_{29}NO_2$.

MW.: 327.46 g/mol.

^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) = 1.71 (d, 3 H, CH_3−CH), 2.63 (t, 4 H, CH_2−CH_2), 4.55 (d, 2 H, CH_2−O), 5.60 (dt, 1 H, CH−CH), 5.72 (dq, 1 H, CH−CH), 6.03 (ddq, 1 H, CH−CH), 6.22 (dd, 1 H, CH−CH).

Reference: Nebhani L., Sinnwell S., Lin C.Y., Coote M.L., Stenzel M.H., Barner-Kowollik C., Journal of Polymer Science: part A Polymer Chemistry **2009**, 47 (22), 6053-6071.

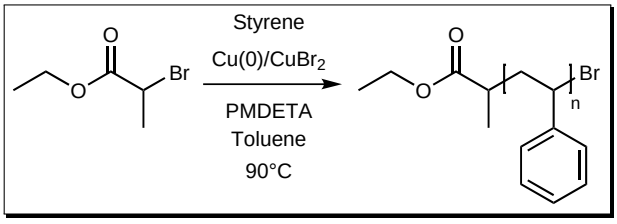
IV.7.3.6 Polyisobornylacrylate-Diene (PiBA-OD)



PiBA-Br (0.468 g, 0.18 mmol, 1 equivalent) was dissolved in dry tetrahydrofuran (THF, 5 mL). Hereby hexadiene-1-ol derivative (**73**, 0.1 g, 0.36 mmol, 2 equivalents) and 1.8 diazobicyclo(5.4.0)undec-7-ene (DBU) (54 mg, 0.036 mmol, 0.2 equivalent) was added at room temperature. The resulting mixture was stirred for 5 days at 50 °C under nitrogen atmosphere. The polymer was then precipitated in cold methanol (25 mL), filtrated, washed thoroughly with methanol and dried overnight in a vacuum oven at 40 °C. Via ^{1}H NMR it was determined that the reaction went to full conversion.

$\boldsymbol{M_n}$ **(SEC)**: 4200 g mol^{-1}, $\boldsymbol{M_w}$ **(SEC)**: 4700 g mol^{-1}, $\boldsymbol{Đ}$ **(SEC)**: 1.20

IV.7.3.7 Polystyrene-bromide (PS-Br)



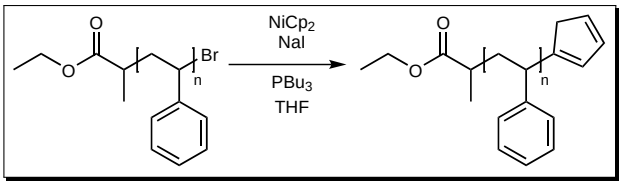
Styrene (5 mL, 43.5 mmol, 50 equivalents), toluene (5 mL), Cu(0) (30 pellets), Cu(II)Br_2 (19.43 mg, 86.9 µmol, 0.1 equivalent) and pentamethyldiethylenetriamine, (PMDETA, 63.6 µL, 0.304 mmol, 0.35 equivalent) were weighed into a flask and degassed for one hour with a continuous nitrogen sparge. ethyl 2-bromopropionate (112.97 µL, 0.87 mmol, 1 equivalent) was degassed separately in an ampule by nitrogen sparge for one hour. After addition of the initiator to the reaction mixture, the flask was placed in an oil bath at 90 °C and the reaction started. After 18 hours, the reaction was stopped (82 % conversion) by cooling in liquid nitrogen under air atmosphere and precipitation in cold methanol (100 mL). The precipitate was filtered off and dissolved in THF (100 mL). The copper catalyst was removed by passing the reaction mixture over a column of Al_2O_3.

After evaporating the excess solvent until a volume of 20 mL, the polymer was precipitated in cold methanol (200 mL). The polymer was filtered, washed with methanol and again dissolved in tetrahydrofuran (THF, 20 mL). Finally the polymer was precipitated in cold methanol (200 mL) and dried overnight in a vacuum oven at 40 °C. Via ^{1}H NMR the end-group fidelity was calculated to be 97%.

$\boldsymbol{M_n}$ **(SEC)**: 4200 g mol^{-1}, $\boldsymbol{M_w}$ **(SEC)**: 4500 g mol^{-1}, $\boldsymbol{Đ}$ **(SEC)**: 1.08

Reference: Tom J., Hornby B., West A., Harrisson S., Perrier S., Polymer Chemistry **2010**, 1 (4), 420-422.

IV.7.3.8 Polystyrene-Cyclopentadiene (PS-Cp)

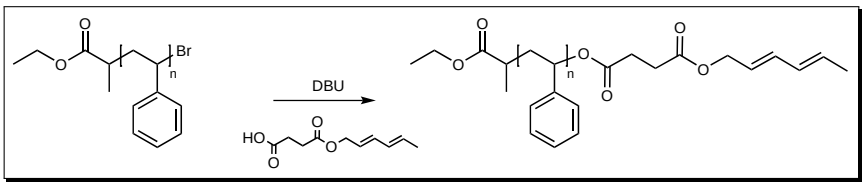


Bromide terminated PS (PS-Br - 0.18 mmol, 1 equivalent) was mixed with tributylphosphine (89 µL, 0.36 mmol, 2 equivalents) and sodium iodide (0.162 g, 1.08 mmol, 6 equivalents) and dissolved in anhydrous tetrahydrofuran (THF, 2 mL). This solution was placed under nitrogen atmosphere. Separately, a stock solution of $NiCp_2$ in dry THF (0.18 mol/L) was prepared under a nitrogen atmosphere. Then the solution of $NiCp_2$ (2 mL) was added to the polymer solution and this mixture was stirred overnight at room temperature. The solution was filtrated over a short column of basis alumina, to remove the precipitated nickel(II)bromide. Then the polymer was precipitated in cold methanol, filtrated and washed thoroughly with methanol. The resulting powder was then dissolved in chloroform and washed three times with distilled water. To obtain the PS-Cp, the polymer was again precipitated in cold methanol, filtrated, washed thoroughly with methanol and dried in a vacuum oven overnight at 40 °C. Via ^{1}H NMR it was determined that the reaction went to full conversion.

$\boldsymbol{M_n}$ **(SEC)**: 4000 g mol^{-1}, $\boldsymbol{M_w}$ **(SEC)**: 4400 g mol^{-1}, $\boldsymbol{Đ}$ **(SEC)**: 1.10

Reference: Inglis A.J.; Paulöhrl T., Barner-Kowollik C. Macromolecules **2009**, 43, 33-36.

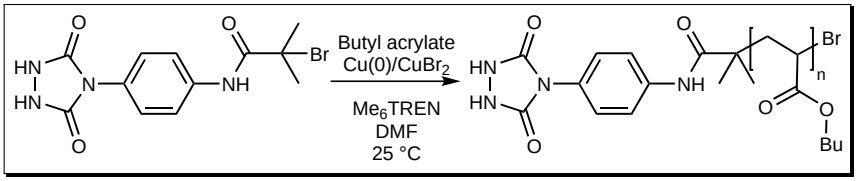
IV.7.3.9 Polystyrene-diene (PS-OD)



PS-Br (0.8 g, 0.18 mmol, 1 equivalent) is dissolved in dry tetrahydrofuran (THF, 5 mL). Hereby hexadiene-1-ol derivative (**73**, 0.1 g, 0.36 mmol, 2 equivalents) and 1.8 diazobicyclo(5.4.0)undec-7-ene (DBU) (54 mg, 0.036 mmol, 0.2 equivalent) is added at room temperature. The resulting mixture is stirred for 5 days at 50 °C under nitrogen atmosphere. The polymer was then precipitated in cold methanol (25 mL), filtrated, washed thoroughly with methanol and dried overnight in a vacuum oven at 40 °C. Via ^{1}H NMR it was determined that the reaction went to full conversion.

$\boldsymbol{M_n}$ **(SEC)**: 3400 g mol^{-1}, $\boldsymbol{M_w}$ **(SEC)**: 3600 g mol^{-1}, $\boldsymbol{Đ}$ **(SEC)**: 1.09

IV.7.3.10 Polybutylacrylate-Urazole (PBA-Urazole)



Butyl acrylate (2.1 mL, 14.66 mmol, 50 equivalents), dimethylformamide (DMF, 4 mL), Cu(0) (20 pellets), urazole-initiator (**56**, 100 mg, 0.29 mmol, 1 equivalent) were weighed into a flask and degassed for one hour with a continuous nitrogen sparge. In a separate vial, Cu(II)Br$_2$ (3.27 mg, 0.29 mmol, 0.05 equivalent), tris[2-(dimethylamino)-ethyl]amine (Me$_6$TREN, 8.1 mg, 0.04 mmol, 0.12 equivalent) and DMF (1.22 mL) were degassed separately for one hour. The reaction was started by the addition of the Cu(II)Br$_2$/ligand-solution to the reaction mixture and the flask was placed in an oil bath at 25 °C. After 22 hours, the reaction was stopped (83 %) by cooling in liquid nitrogen under air atmosphere and removing the copper catalyst by passing the reaction mixture over a column of Al_2O_3. After evaporating the excess solvent, the polymer was poured in a petri dish and dried overnight in a vacuum oven at 40 °C.

$\boldsymbol{M_n}$ **(SEC)**: 20 000 g mol^{-1}, $\boldsymbol{M_w}$ **(SEC)**: 27 700 g mol^{-1}, $\boldsymbol{Đ}$ **(SEC)**: 1.38

IV.7.3.11 General procedure for oxidizing urazole polymers

1 mmol of polymer with an urazole end group is dissolved in 5 mL of dichloromethane. Hereby 0.3 mmol of DABCO-Br is added at room temperature. The solution was allowed to stir for three hours at room temperature. Then the solution was filtrated and concentrated *in vacuo* to obtain the polymer with a TAD end group.

IV.7.3.12 General procedure for polymer-polymer conjugation

The polymer (50 mg, 1 equivalent) with an Cp/Indole end group was dissolved in tetrahydrofuran (THF, 0.5 mL). Hereby a solution of TAD polymer in THF (0.5 mL,1 equivalent) was added at room temperature. The solution was allowed to stir until the red colour disappeared. The obtained block copolymer was precipitated in the appropriate solvent, filtrated, washed thoroughly and dried overnight in a vacuum oven at 40 °C.

IV.7.3.13 Synthesis of a polyurethane network

To a solution of 2,4-hexadiene-1,6-diol (HDD, 0.244 g, 2.14 mmol,0.68 equivalent) in dimethylformamide (3 mL) was added 4,4'-(4,4'-diphenylmethylene)-bis-(1,2,4-triazoline-3,5-dione) (0.194 g, 0.536 mmol, 0.17 equivalent). After the reaction had gone to completion (less than one minute, disappearance of the red colour), the solution was mixed with polypropylene oxide (2 g, 1 mmol, 0.32 equivalent) and hexamethylene diisocyanate (0.529 g, 3.15 mmol, 1 equivalent) in a 50 mL flask. Then dibutyltindilaurate (29 µL) was added and the mixture was well stirred. This reactive mixture was injected between two glass plates, separated by silicone spacers and placed in an oven at 70 °C for six hours. The obtained network was dried overnight in a vacuum oven at 40 °C.

T_g (DSC): −50 °C, **T_{deg} (TGA)**: 300 °C.

IV.8 Bibliography

[1] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angewandte Chemie International Edition* **2001**, *40*, 2004–2021.

[2] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angewandte Chemie International Edition* **2002**, *114*, 2708–2711.

[3] P. Espeel, F. E. Du Prez, *Macromolecules* **2015**, *48*, 2–14.

[4] C. J. Hawker, V. V. Fokin, M. G. Finn, K. B. Sharpless, *Australian Journal of Chemistry* **2007**, *60*, 381–383.

[5] C. Barner-Kowollik, F. E. Du Prez, P. Espeel, C. J. Hawker, T. Junkers, H. Schlaad, W. Van Camp, *Angewandte Chemie International Edition* **2011**, *50*, 60–62.

[6] C. W. Tornøe, C. Christensen, M. Meldal, *The Journal of Organic Chemistry* **2002**, *67*, 3057–3064.

[7] M. Meldal, *Macromolecular Rapid Communications* **2008**, *29*, 1016–1051.

[8] T. R. Chan, R. Hilgraf, K. B. Sharpless, V. V. Fokin, *Organic Letters* **2004**, *6*, 2853–2855.

[9] C. E. Hoyle, T. Y. Lee, T. Roper, *Journal of Polymer Science Part A: Polymer Chemistry* **2004**, *42*, 5301–5338.

[10] C. Hoyle, C. Bowman, *Angewandte Chemie International Edition* **2010**, *49*, 1540–1573.

[11] M. A. Tasdelen, *Polymer Chemistry* **2011**, *2*, 2133–2145.

[12] R. Gostl, A. Senf, S. Hecht, *Chemical Society Reviews* **2014**, *43*, 1982–1996.

[13] T. Gruendling, K. K. Oehlenschlaeger, E. Frick, M. Glassner, C. Schmid, C. Barner-Kowollik, *Macromolecular Rapid Communications* **2011**, *32*, 807–812.

[14] M. Dietrich, G. Delaittre, J. P. Blinco, A. J. Inglis, M. Bruns, C. Barner-Kowollik, *Advanced Functional Materials* **2012**, *22*, 304–312.

[15] T. Pauloehrl, G. Delaittre, V. Winkler, A. Welle, M. Bruns, H. G. Börner, A. M. Greiner, M. Bastmeyer, C. Barner-Kowollik, *Angewandte Chemie International Edition* **2012**, *51*, 1071–1074.

[16] C.-H. Wong, S. C. Zimmerman, *Chemical Communications* **2013**, *49*, 1679–1695.

[17] P. Espeel, F. Goethals, F. E. Du Prez, *Journal of the American Chemical Society* **2011**, *133*, 1678–1681.

[18] A. Gandini, *Progress in Polymer Science* **2013**, *38*, 1–29.

[19] T. Maeda, H. Otsuka, A. Takahara, *Progress in Polymer Science* **2009**, *34*, 581–604.

[20] A. Wilson, G. Gasparini, S. Matile, *Chemical Society Reviews* **2014**, *43*, 1948–1962.

[21] A. J. Inglis, M. H. Stenzel, C. Barner-Kowollik, *Macromolecular Rapid Communications* **2009**, *30*, 1792–1798.

[22] C. F. Hansell, P. Espeel, M. M. Stamenović, I. A. Barker, A. P. Dove, F. E. Du Prez, R. K. O'Reilly, *Journal of the American Chemical Society* **2011**, *133*, 13828–13831.

[23] S. Billiet, K. De Bruycker, F. Driessen, H. Goossens, V. Van Speybroeck, J. M. Winne, F. E. Du Prez, *Nature Chemistry* **2014**, *6*, 815–821.

[24] H. Ban, J. Gavrilyuk, C. F. Barbas, *Journal of the American Chemical Society* **2010**, *132*, 1523–1525.

[25] A. J. Inglis, T. Paulohrl, C. Barner-Kowollik, *Macromolecules* **2009**, *43*, 33–36.

[26] Z. Wang, L. Yuan, N. M. Trenor, L. Vlaminck, S. Billiet, A. Sarkar, F. E. Du Prez, M. Stefik, C. Tang, *Green Chemistry* **2015**, *17*, 3806–3818.

[27] B. Vonhören, O. Roling, K. De Bruycker, R. Calvo, F. E. Du Prez, B. J. Ravoo, *ACS Macro Letters* **2015**, 331–334.

[28] S. van der Heijden, K. De Bruycker, R. Simal, F. Du Prez, K. De Clerck, *Macromolecules* **2015**, *48*, 6474–6481.

Abstract:

With the aid of triazolinedione (TAD) chemistry, an additive-free, straightforward functionalization and crosslinking strategy for numerous plant oils was developed. In a first step, model studies on the most common natural fatty acids were performed with the aid of monofunctional TAD moieties. These equimolar functionalization reactions were monitored by NMR and MS analysis, further facilitated by the disappearance of the characteristic red colour of the TAD molecule. Then, a series of synthesized, bifunctional TAD molecules were used for the chemical crosslinking of crude plant oils, a process that was typically finished within minutes. In this way, a large variety of plant oil-based foils could be obtained, showing a wide range of thermal properties, which can be tuned and rationalized by the chemical structure of the plant oils used.

Reference:

Türünç O., Billiet S., De Bruycker K., Ouardad S., Winne J.M., Du Prez F.E., *European Polymer Journal* **2015**, *65*, 286-297.

Chapter V

Application of irreversible TAD *click* chemistry for the synthesis of plant oil based materials

V.1 Introduction

When introducing a 'new' *click* chemistry platform, it is of importance to implement it in several domains to demonstrate its versatility. In chapter IV, the irreversible TAD chemistry was introduced where dienes or *enes* are used as an efficient partner for TAD. However, all involved (di)enes were of synthetic origin. In our quest to find additional suitable partners for this irreversible reaction, our attention was quickly drawn to natural products, more specifically plant oils. Plant oils contain unsaturations that are not easy to functionalize, thus this seemed a challenging starting point to explore the newly developed chemistry in this area of renewable chemicals.

One of the most discussed topics of the last years, both on academic and industrial level, is the introduction of the 'green chemistry' concept, and consequently the 'green chemistry's twelve principles'.[1] These guidelines challenged researchers and companies to both revise their ongoing processes and to plan the future processes in a more sustainable way. Considering the total plastics production worldwide – annually about 300 million tons – it seems logical to implement these concepts in polymer chemistry as fast as possible.[2] One possibility is the substitution of the starting components based on fossil resources,

by their renewable counterpart. It has already been shown that oils and fats of vegetable and animal origin have a great potential to be exploited as building blocks for renewable plastics.[3–6] Vegetable oils are transformed by classical and well-established oleochemical reactions to produce either free fatty acids and glycerol (by hydrolysis) or fatty acid methyl esters (by transesterification).[7] In a second step, these adducts can be hydrogenated to fatty alcohols or be transformed to fatty amines in industrial scale[8], which are utilized as surfactants[9,10] or in lubricants[11,12] and coatings.[13] Moreover, it is important to mention that fatty acid methyl esters have been of great interest in the last decade due to the increasing biodiesel production.[14]

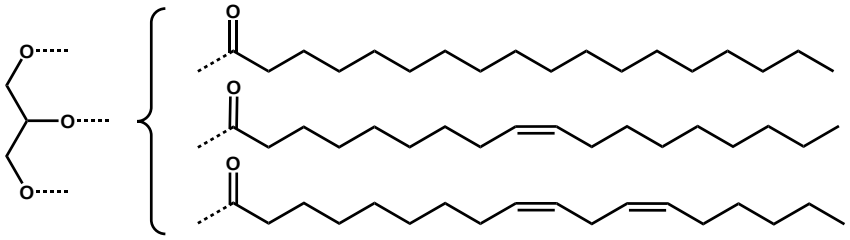


Figure V.1: Chemical structure of a triglyceride, consisting out of a combination of saturated, monounsaturated or polyunsaturated fatty acids.

When looking to the chemical structure of vegetable oils (see Figure V.1), it becomes clear that they contain a large number of unsaturations. These double bonds could be used for further chemical modification of the oils to obtain novel compounds and materials. However, these unsaturations offer a rather limited scope for direct chemical transformations. Although some classical reactions are known to utilize these double bonds efficiently (f.e. hydrogenation[15], ozone cleavage[16], hydroformylation[17] and epoxidation[10]), these transformations typically require transition metal catalysts, and/or a precise control of reactions conditions, making them not readily accessible.

Recently, Mecking *et al.* applied a pincer catalyst to synthesize linear α-ω-diesters (and their diol derivatives) from monounsaturated fatty acids, with an efficiency that allowed the direct polycondensation of these products without further purification, yielding high molecular weight polyesters.[18] Meier's group reported on the olefin metathesis and thiol-*ene* addition reactions as efficient reaction pathways to synthesize renewable building blocks and polymers from 10-undecenoic acid, a semisynthetic fatty acid with a terminal olefin derived from castor oil.[19] However, these mild chemistries are not that straightforward for more common natural fatty acids that have internal unsaturations: olefin

metathesis results here in the formation of a stoichiometric amount of by-products[20], while the thiol-*ene* addition reaction results in extremely decreased reaction efficiency.[21]

In oleochemistry, the interest for simple and effective reactions started to grow, leading to the preparation of renewable polymers from fatty acids and plant oils. As discussed above, the thiol-*ene* addition reaction has been applied to plant oils, yet its convenient *click* character remained limited to the unnatural 10-undecenoic acid. On the other hand, Petrovic *et al.* reported on the successful exploration of solvent-and catalyst-free azide-alkyne cycloaddition on various plant oils.[22] In their studies, they first prepared azidated and alkynated plant oils, and subsequently crosslinked these adducts with one another. A similar strategy, yet with a different chemistry was followed by Gandini and co-workers applying Diels–Alder reactions, in which the thermal reversibility character of the retro-Diels–Alder reactions between furan and maleimide-functionalized adducts enabled the authors to obtain thermo-reversible renewable materials.[23]

As already discussed in Chapter II, TAD compounds are known to be one of the strongest *ene*- and *diene*-ophiles in organic chemistry. However, due to the limited availability and perhaps its exotic reputation, the reactive molecules were never adopted in oleochemistry. An example of a niche-type application of TAD chemistry is a sample-pre-treatment method to facilitate the identification of the different isomers of conjugated fatty acids in plant oils via GC–MS[24]. A first application of TAD chemistry for plant oil derived materials was very recently reported by Biswas *et al.*, in which soybean oil was modified with readily available 4-phenyl-1,2,4-triazoline-3,5-dione (PhTAD (**1**)) and shown to be applicable as lubricants and/or thickeners.[25]

In this chapter, the use of TAD chemistry in combination with plant oils for the additive-free preparation of polymer materials is described. In a first step, model studies on the most common natural fatty acids will be described. These equimolar functionalization reactions can be readily monitored by NMR and MS analysis, further facilitated by the disappearance of the characteristic red colour of the TAD molecule. Then, a series of synthesized, bifunctional TAD molecules will be used for the chemical crosslinking of crude plant oils. In this way, a large variety of polymer networks will be obtained and fully characterized.

V.2 Model study with fatty acid derivatives

Before TAD reactions were performed directly on the crude plant oils, an extensive model study was conducted in order to be able to understand the reactions that can happen in a more complex setting. Thus, a range of isolated mono- and polyunsaturated fatty acids (or derivatives thereof) were combined with monofunctional TAD moieties under simple reaction conditions (at ambient temperature, no inert atmosphere). In this way the relative reactivity and reaction rates of the various olefins found in fatty acids can be revealed. Hence, oleyl alcohol (**74**), elaidyl alcohol (**75**) / methyl elaidate (**76**), methyl ricinoleate (**77**), 10-undecenoic acid (**78**), methyl linoleate (**79**) and methyl linolenate (**80**) were mixed with PhTAD (**1**) in equimolar amount (for the chemical structures of the fatty acid derivatives see Figure V.2).

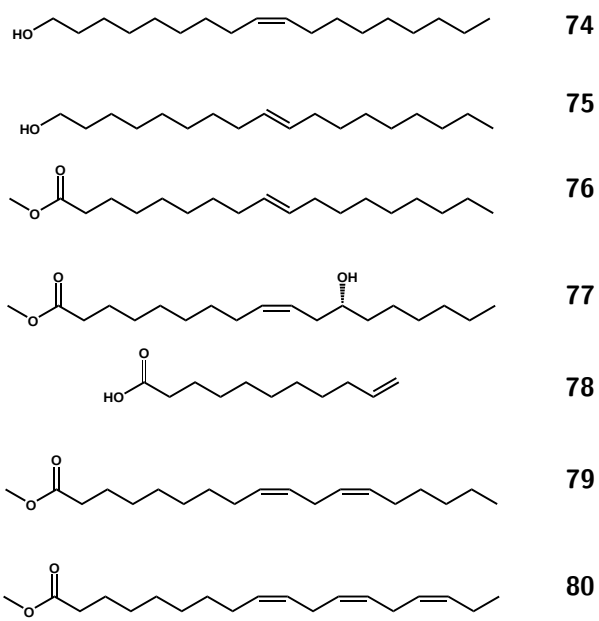


Figure V.2: Chemical structures of used fatty acid derivatives

First, the reaction between PhTAD (**1**) with oleyl alcohol (**74** - one of the most common lipid tails in plant oils) was studied in detail by NMR (see Figure V.3). Full conversion was observed within 10 minutes and, as can be seen in the corresponding ^{1}H NMR and MS spectrum, the resulting reaction mixture essentially only contains the expected *ene*-adduct (**81**). The reaction mechanism is formally a concerted Alder-*ene* reaction (for a general mechanism see chapter II), but in fact a stepwise process takes place in which the π-electrons of the olefin combine with the low-energy *LUMO* in the triazolinedione

π-MO-system. This results in the fast formation of a zwitterionic aziridinium adduct with one of the unsaturated nitrogen atoms, which then results in the abstraction of the allylic proton by the remaining, negatively charged nitrogen, giving a new olefinic bond in the final neutral adduct.

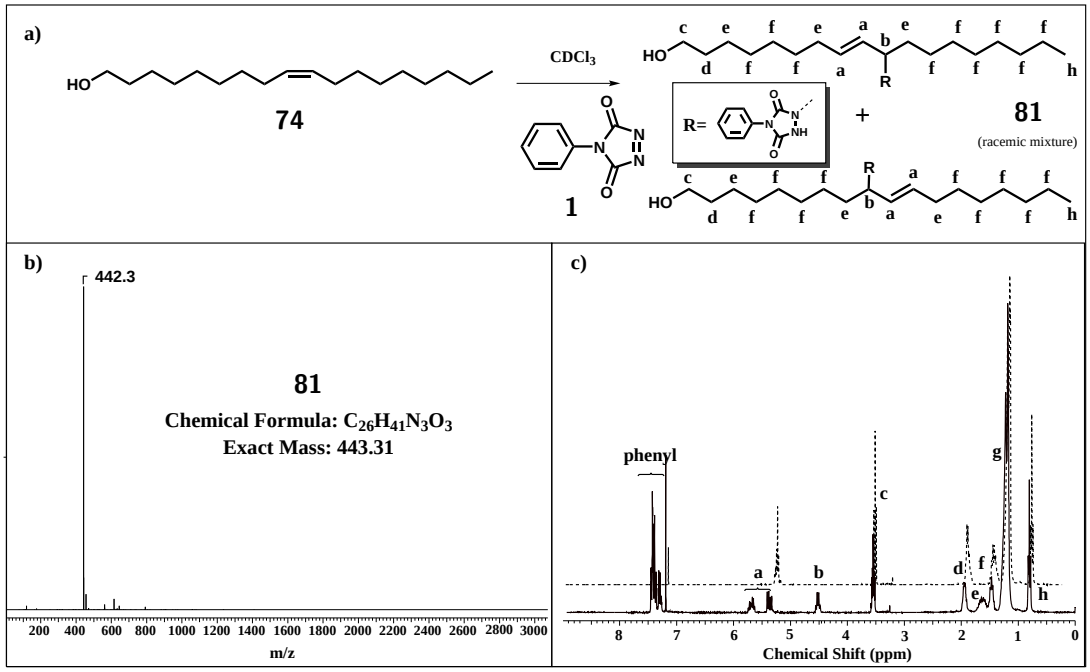


Figure V.3: a) Reaction scheme of the reaction in $CDCl_3$ of oleyl alcohol (**74**) in equimolar amount to PhTAD (**1**), b) corresponding MS spectrum (ESI – negative mode) and c) ^{1}H NMR with dashed line representing oleyl alcohol (**74**) and full line 1:1 adduct of oleyl alcohol and PhTAD (**81**).

Due to the fact that the carbon–carbon double bond is not consumed in the Alder-*ene* reaction, but rather shifts along the alkyl chain, two positional isomers can be formed. Therefore, the formed product is in fact a mixture of two closely related compounds, in which the urazole moiety is located on either the *C9* or *C10* position. As there is no steric or electronic bias towards either side of the initial olefinic bond in oleyl alcohol, both isomers are formed in equal amount. The MS spectrum of the crude reaction (Figure V.3b) clearly reveals that a second reaction, with the newly formed alkene, does not occur when only one equivalent of PhTAD (**1**) is used. In Figure V.3c, no trace of the characteristic olefinic proton resonances of oleyl alcohol (5.4 ppm) can be observed, demonstrating the full conversion.

Having established an efficient and quantitative *click* reaction between PhTAD (**1**) and oleyl alcohol under equimolar conditions, the next step was to explore the reaction of the

resulting adduct with a second equivalent of the TAD compound. Addition of a second equivalent of PhTAD to the freshly prepared adduct, resulted in a slow fading of the red colour of the TAD reagent. This red colour disappeared in a matter of hours rather than minutes, indicating a much slower reaction. ^{1}H NMR and MS analysis of the solution demonstrated that the reaction again underwent a quantitative *ene* reaction (leading to a mixture of diastereomers), resulting in the incorporation of a second urazole moiety (**82** - see Figure V.4). The prolonged reaction time can be easily rationalized by the increased steric hindrance around the reacting olefin, the E(*trans*)-configuration of the alkene bond, and the electron withdrawing properties of the urazole (nitrogen) substituent, all resulting in a less reactive olefin. This marked difference in reactivity between the first and second reaction can thus be exploited to introduce multiple and distinct functionalities in a site-controlled manner, and for fine-tuning of the crosslinking kinetics of olefinic monomers.

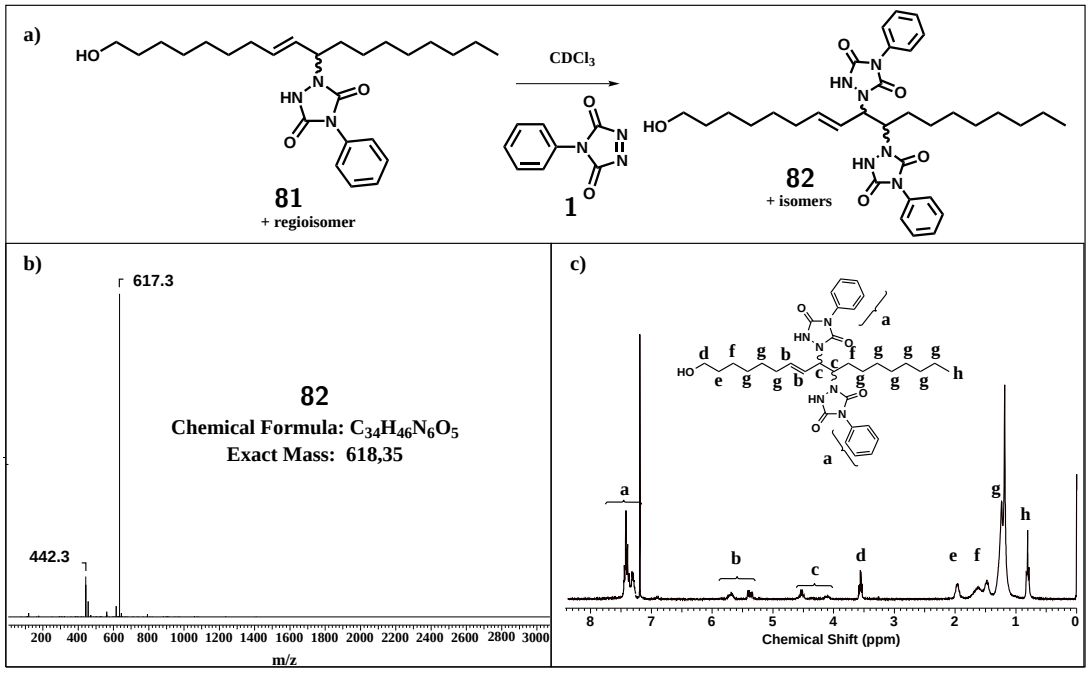


Figure V.4: a) Reaction scheme of the reaction of 1:1 adduct of oleyl alcohol and PhTAD (**81**) in equimolar amount to PhTAD (**1**), b) corresponding MS spectrum (ESI – negative mode) and c) ^{1}H NMR spectrum of the 2:1 adduct of PhTAD and oleyl alcohol (**82**).

Next to oleyl-derivatives, other monofunctional fatty acid derivatives were tested for their reactivity towards TAD moieties. Most of the tested substrates (**76**, **77**, **78**, **79** and **80**) resulted in highly specific and quantitative adduct formations, confirming the *click*-nature of TAD-based *ene*-reactions.

Conveniently, all unsaturated plant oils exclusively contain Z(*cis*)-double bonds in their natural form. In order to better appreciate the known reactivity difference between *cis* and *trans*-double bonds, we prepared the *trans*-analogue elaidyl alcohol (**75**) from oleyl alcohol via a simple mercaptan-mediated isomerization.[26] The equimolar reaction of elaidyl alcohol with PhTAD needed about 35 min for the red colour to fade (compared to 10 min for the *cis*-analogue). This classical reactivity difference is easily rationalized by the lower stability of *cis*-alkenes.[27]

Ricinoleic acid methyl ester (**77**), the major fatty acid constituent of castor oil with a lipid tail differing from that of oleyl alcohol by having an additional hydroxyl group on *C12*, has also been checked for its reactivity with TAD. Surprisingly, the reaction with PhTAD proceeded even slower than with the trans oleyl alcohol (120 min). This result indicates the relative importance of proximal electron withdrawing groups.

In order to further establish the effect of the nature of the double bond of fatty acids on a TAD reaction, a *C11* building block with a terminal double bond derived from castor oil (10-undecenoic acid, **78**) was tested (see Figure V.5). Despite the fact that 10-undecenoic acid underwent a quantitative equimolar reaction with PhTAD, the colour disappeared

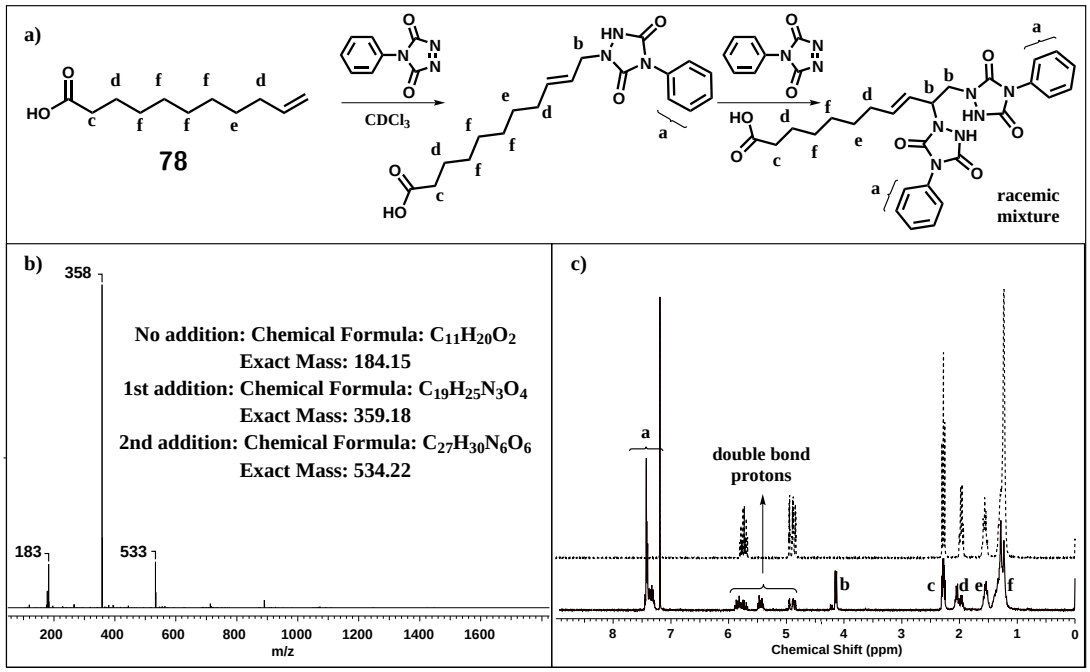


Figure V.5: a) Reaction scheme of the reaction of 10-undecenoic acid (**78**) in 1:1 molar ratio to PhTAD, b)corresponding MS spectrum (ESI – negative mode) and c) ^{1}H NMR with dashed line representing 10-undecenoic acid and full line the 1:1 adduct of 10-undecenoic acid and PhTAD.

only after 8 hours at room temperature. This is consistent with the poor electronic activation of a terminal olefin. However, careful analysis of the ^{1}H NMR spectrum of the reaction mixture showed that most of the terminal double bonds were still present, and thus most of 10-undecenoic acid had actually not reacted with the TAD reagent (see Figure V.5). Nevertheless, new vinyl proton signals with higher shifts were formed, which revealed a more rapid second reaction of the TAD compound with the initially formed mono-addition products. Apparently, even though the product formed after the first reaction has a low reactivity towards TAD compounds due to the reasons discussed earlier, this reactivity is still higher than that of the terminal alkene itself. In another trial, where two equivalents of PhTAD were used, a complete conversion was not obtained. This unnatural fatty acid thus lacks *click-like* behaviour when combined with TAD molecules.

The obtained results for the TAD reaction with a range of mono-unsaturated fatty acid derivatives at ambient conditions can be readily interpreted by comparing with the values on the kinetics of TAD chemistry with common olefins described in Chapter III. This reactivity order clearly shows that a higher substitution degree of the alkene increases the reactivity over several orders of magnitude. Moreover, having the double bond at a terminal position indeed strongly decreases the reactivity. As expected, these reactivity trends are in line with those observed for the tested simple mono-unsaturated fatty acid derivatives (see Table V.1).

Table V.1: Fatty acid derivatives and their respective reaction time (min) with PhTAD

Fatty acid derivative	**Reaction time (min)**
Oleyl alcohol	10
Elaidic alcohol	35
Ricinoleic acid	120
Undecenoic acid	480

In order to get a more relevant picture for the case of natural plant oils, and to perform a systematic overview of the most common fatty acids, the above simple reactivity tests were also performed on polyunsaturated fatty acids. From this extended study, it could be seen that methyl linoleate (**79** - see Figure V.6) and methyl linolenate (**80**) also reacted quantitatively and specifically. For methyl linoleate the characteristic colour of TAD disappeared after 1 min, while for methyl linolenate the reaction already went to completion in less than 40 sec. This significant increase in reactivity as compared

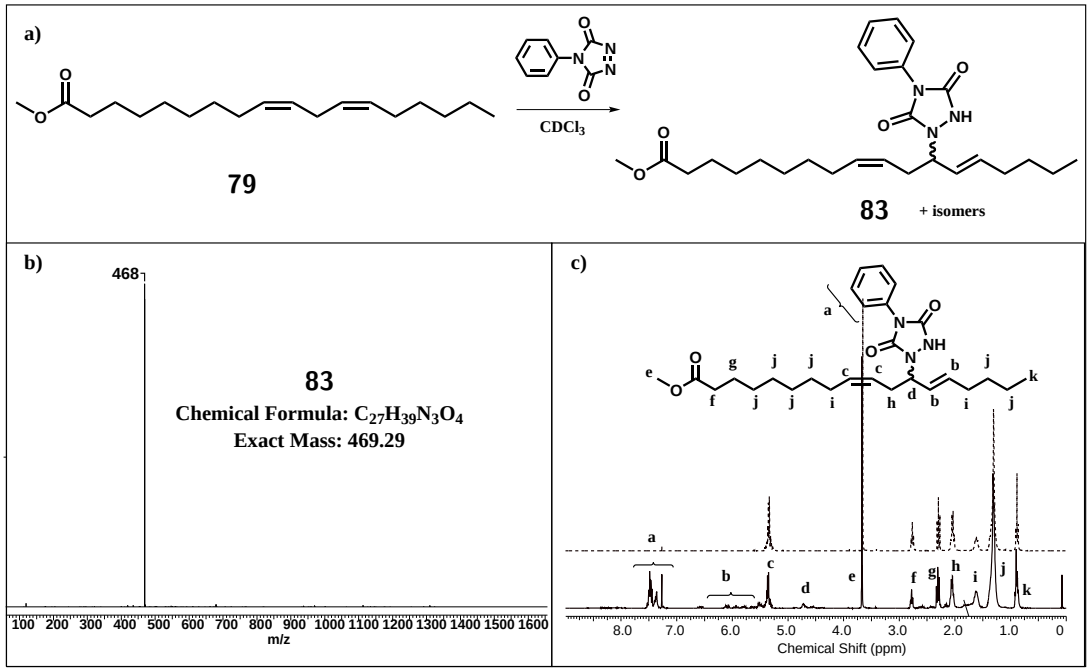


Figure V.6: a) Reaction scheme of the reaction of methyl linoleate (**79**) in 1:1 molar ratio to PhTAD, b) corresponding MS spectrum (ESI – negative mode) and c) ^{1}H NMR with dashed line representing methyl linoleate and full line the 1:1 adduct of methyl linoleate and PhTAD (**83**).

to monounsaturated fatty acids is most likely found in the homoconjugated nature of these polyunsaturated systems. While a nearby olefin can be regarded as an electron-withdrawing substituent, this effect appears to be compensated by a higher reactivity of the π-electrons. Indeed, the neighbouring π-systems in a homoconjugated system can influence each other by secondary orbital interaction, both in the ground state and the transition states, leading to much faster orbital-controlled reactions with the electron deficient TAD moieties by neighbouring group participation. It is important to note that the second reaction of TAD with the remaining *cis*-olefin does not seem to compete with the reaction of the initial homoconjugated poly*ene* system, opening further possibilities for specific and site-directed bis-functionalizations.

In an attempt to further establish the utility of the above described TAD-based functionalization, we considered the possible applications of this chemistry for the synthesis of building blocks from fatty acids. Thus, we briefly explored the possibility for further selective transformations of the obtained allylic urazoyl products. Since plant oils are readily reduced in a catalytic hydrogenation, we subjected the 1:1 adduct of oleyl alcohol and PhTAD to a similar procedure (see V.6.3.5). ^{1}H NMR analysis of the resulting

reaction product confirmed the clean formation to the hydrogenated adduct (**84** - see Figure V.7) as well as the presence of the urazole moiety.

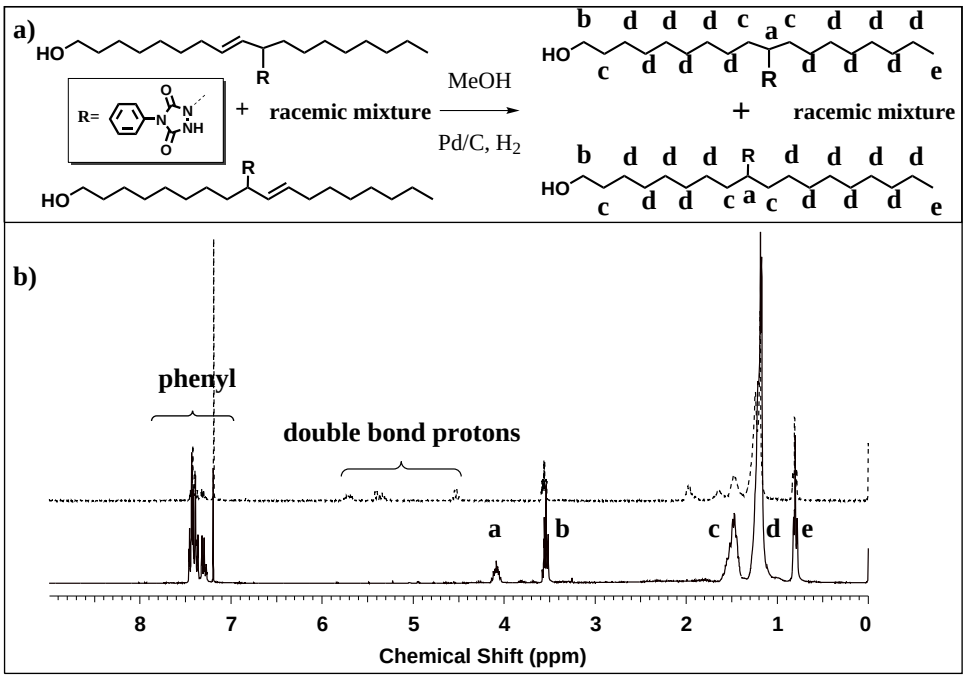


Figure V.7: a) Reaction scheme of the hydrogenation of the 1:1 adduct (**81**) of oleyl alcohol and PhTAD and b)^{1}H NMR spectra representing 1:1 adduct (**81**) of oleyl alcohol and PhTAD (dashed line) and the hydrogenated product (**84** - full line).

Finally, in order to demonstrate that these TAD-based *click* reactions can also be used for specific post-modifications and functionalization, a TAD molecule containing an ATRP initiator was added to oleyl alcohol (see **85** in Figure V.8). Both ^{1}H NMR and MS analysis showed again a clean and complete conversion (see Figure V.8). These results clearly show that TAD-based chemistry can be implemented to introduce functionalities onto fatty acids and derivatives in a quite efficient way.

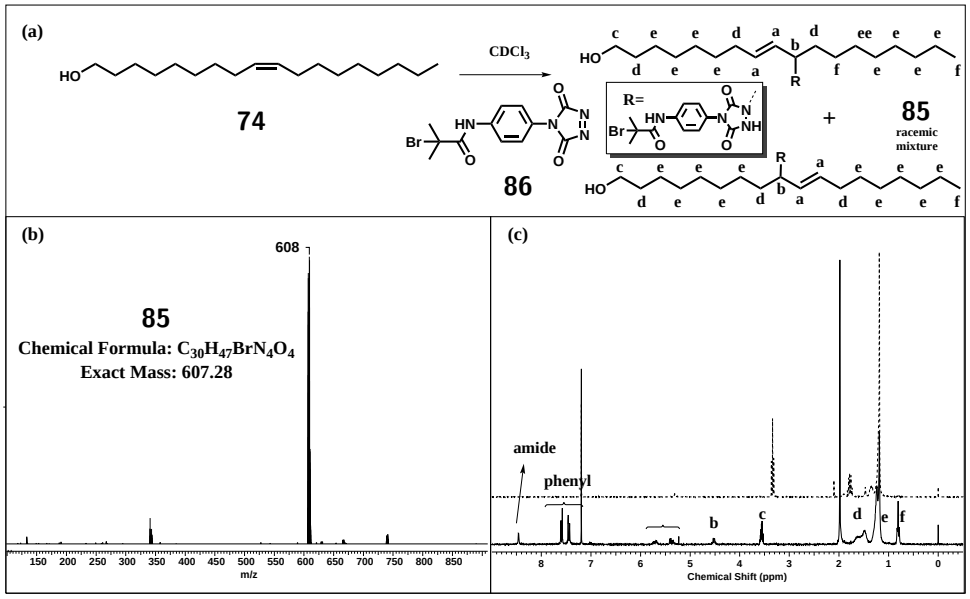


Figure V.8: a) Reaction scheme of the reaction of oleyl alcohol (**74**) in equimolar amount to ATRP-TAD (**86**), b) corresponding MS spectrum (ESI – negative mode) and c) ^{1}H NMR with dashed line representing oleyl alcohol (**74**) and the full line the 1:1 adduct of oleyl alcohol and ATRP-TAD (**85**).

V.3 Direct crosslinking of plant oils

Having established the reactivity of TAD reagents with most natural fatty acids, the direct crosslinking of a range of commercially available plant oils with an aromatic TAD crosslinker (4–4'-methylenediphenyldi-1,2,4-triazoline-3,5-dione, MDI-TAD **9**) was tested at ambient conditions. The oils tested were olive, sunflower, colza, corn, groundnut, pumpkinseed, soybean, and castor oils (see Figure V.9a). None of the oils tested in this study were subjected to any pretreatment and reactions have been performed under air. When considering the curing behaviour of the aforementioned plant oils, it became apparent that a direct correlation between the curing kinetics and plant oils composition is very difficult due to the dependence on a number of parameters, such as molar equivalence of reactants, saturated and unsaturated fatty acid amount and composition, number of free fatty acids, and diglycerides. Since most of these parameters cannot be controlled, the choice was made to limit the gelation kinetics of natural products to a qualitative study.

In order to estimate the amount of bisTAD needed to crosslink all plant oils, the total number of *enes* per gram of castor and soybean oil was quantified by proton NMR analysis with 1-hexene as an internal standard. In order to obtain a theoretical TAD-*ene* molar

equivalence, the mass ratios of oil:crosslinker for these selected oils were found to be 1:1.1 and 1:1.4 respectively. Since a variation of the amount of aromatic crosslinker (**9**) for the different oils will also have a direct influence on the thermal properties of the resulting networks (*vide infra*), we limited these initial studies across different plant oils by using a fixed mass ratio rather than a theoretical TAD-*ene* molar ratio. Therefore, in this study, solutions of plant oils and bisTAD in acetone were mixed in an open flask at room temperature in order to obtain a mixture with a 1:1 mass ratio of oil:crosslinker. Because an *ene* function actually results in another reactive *ene* function after a reaction with TAD, striving for a 1:1 *ene*:TAD molar ratio for a given oil is not very useful anyway, and will each time result in a quite different macromolecular architecture with widely varying properties. At least with a fixed mass ratio, the chemical composition of the copolymer networks can be controlled on the level of constituents, because this ratio translates almost directly into the monomer ratio in the resulting copolymers. This ratio will also most closely correlate to the actual cross-link density in the complex networks (each TAD will always be crosslinked to two triglyceride side chains). Conversely, using molar ratios, the cross-link density would be completely controlled by the composition of the plant oils.

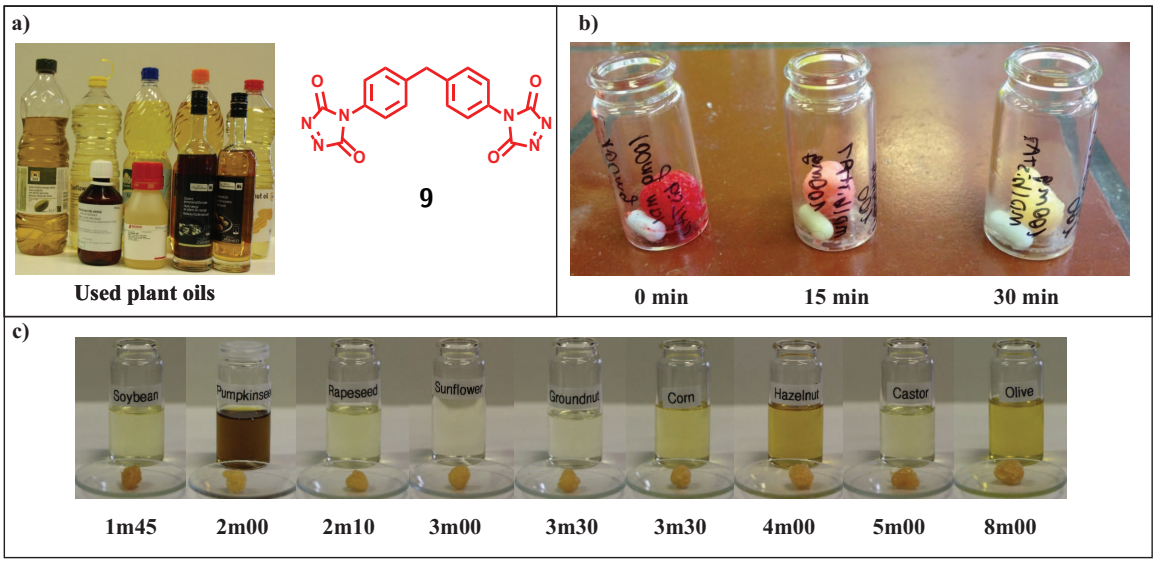


Figure V.9: a) The plant oils used in this study and the used aromatic TAD crosslinker (**9**) for the network formation, b) discolouring after network formation and c) the plant oils and corresponding networks with their respective gelation times (in acetone).

Although gelation occurs swiftly in a period of 2 to 8 min, as expected by the high reactivity of the crosslinker towards the unsaturated fatty acid residues in triglycerides,

the initial red colour of the TAD reagent persists for a much longer time, clearly indicating that the conversion of the TAD reagents was not yet complete. Following initial gelation, the sample was left to cure, which was easily monitored by the slow disappearance of the red colour (see Figure V.9b). For instance, soybean oil showed a gelation time of 1 min and 45 s, while 30 min were needed for full decolouration.

The variation in observed gelation times, ranging from less than 2 min for soybean oil to 8 min for olive oil (see Figure V.9c), can to some extent be correlated to the relative reaction kinetics of different fatty acids as addressed in the model study. Indeed, a higher content of polyunsaturated fatty acids (e.g. linoleic and linolenic acids) for a certain oil (see Table V.2) generally results in a faster gelation. However, the gelation time will also be critically dependent on the *ene*:TAD equivalence (see above) and the average functionality of the oil monomers, i.e. the number of saturated fatty acids and the ratio of free fatty acids, di- and triglycerides. Since those variables cannot be fixed in a study of natural plant oils, it is not really possible to fully rationalize the varying gelation times at this time.

Table V.2: Percentage of mono- and polyunsaturations of plant oils used.[3] The remaining percentages depict the amount of saturated fatty acids.

	% of monounsaturation	**% of polyunsaturation**
Castor	93	7
Olive	78	7
Hazelnut	74	16
Rapeseed	62	30
Groundnut	55	27
Corn	30	57
Sunflower	27	62
Pumpkin	25	57
Soybean	23	61

V.4 Characterization of plant oil networks

In order to establish the thermal properties, the networks prepared were subjected to *differential scanning calorimetry* (DSC) as well as to *thermogravimetric analysis* (TGA).

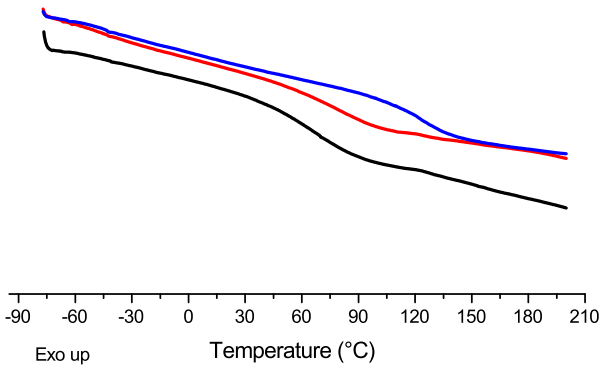


Figure V.10: DSC spectra (2nd heating) of networks of respective olive (black), groundnut (red) and pumpkinseed (blue) oil with bisTAD in a 1:1 weight ratio.

Figure V.10 depicts some examples of the typical thermograms obtained from DSC analysis. As shown, the samples exhibited broad glass transitions (T_g). Therefore, we found it necessary to calculate both onset and midpoint T_g temperatures for a better comparison, which are summarized in Table V.3.

Table V.3: T_g (onset and midpoint) of the networks obtained by combining the respective oil with bis-TAD in a 1:1 weight ratio.

	T_g (Onset, °C)	**T_g (Mid, °C)**
Castor	48	75
Olive	55	60
Hazelnut	51	72
Rapeseed	55	72
Groundnut	59	85
Corn	75	106
Sunflower	27	62
Pumpkin	109	124
Soybean	94	119

When the midpoint T_g's are compared, it can be seen that a T_g-range from 60 up to 124 °C was obtained for the different plant oils. These relatively high glass transition temperatures reflect the rigid structure of the added aromatic bisTAD crosslinker. Obviously, the diverse fatty acid composition of the plant oils resulted in such differences in T_g. A clear correlation of the unsaturation degree of the plant oils and the T_g's of their networks can

be observed (see Figure V.11). This result may be attributed to the fact that polyunsaturated fatty acids are more likely to react twice with a TAD crosslinker on the same lipid tail, which directly affects the chain mobility between two rigid crosslinker moieties. It is important to note that castor oil has a different fatty acid composition compared to the other oils as it is mainly composed of ricinoleic acid that has an alcohol group on *C12*. The difference in T_g of castor oil and that of olive oil with similar percentage of oleic acid can be explained by considering the presence of the alcohol group. Indeed, the alcohol groups of ricinoleic acids contribute to the intermolecular chain interactions in the castor oil network, resulting in a slightly higher T_g. While this can be used to select the plant oil composition as a function of the desired T_g for the corresponding crosslinked material, we anticipated that more T_g variations could also be obtained by starting from aliphatic crosslinkers or by varying the amount of crosslinker.

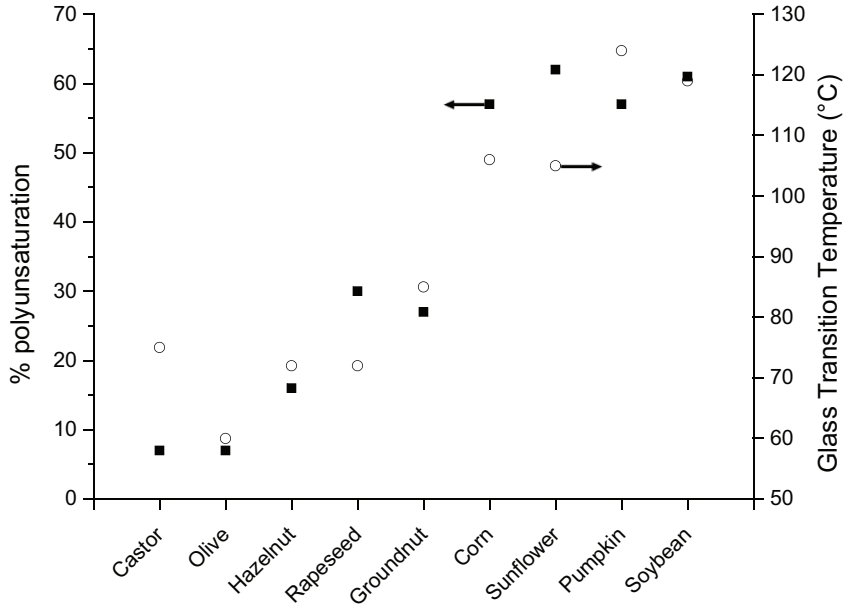


Figure V.11: Representation of the different plant oils used in the crosslink experiment with their percentage of polyunsaturation and respective glass transition temperature (T_g). (Oil:bisTAD (**9**) = 1:1)

In this respect, the amount of crosslinker (**9**) was changed for the pumpkinseed oil network in order to check the influence on the characteristics of the obtained gel. For this, the same gelation experiment was conducted with the exception that the weight ratio plant oil:bisTAD was changed from 1:1 to both excess plant oil (4:3) and excess bisTAD (3:4). With higher amount of the aromatic crosslinker, the T_g of the material increased as expected (see Table V.4). On the other hand, for lower amounts of the crosslinker, a

significant decrease in T_g-value was observed (see Table V.4). Secondly, the use of an aliphatic crosslinker (based on isophorone diisocyanate (IPDI) - **87**) in a 1:1 mass ratio with pumpkinseed oil, also resulted in a clear drop in T_g (64 °C).

Thus, a high variety in T_g (over 70 °C) for these plant oil based materials can be obtained both by changing ratio plant oil:crosslinker as well as the chemical nature of the crosslinker, making this a powerful way to tune the mechanical properties of the obtained materials.

Table V.4: T_g (onset and midpoint) of the networks obtained by combining pumpkinseed oil with an aromatic crosslinker (4) in a 4:3 (excess oil); 1:1 and a 3:4 (excess TAD) weight ratio and combining pumpkinseed oil with an aliphatic crosslinker (14) in 1:1 weight ratio.

	T_g (Onset, °C)	**T_g (Mid, °C)**
4:3	48	66
1:1 (MDI)	109	124
1:1 (IPDI)	36	64
3:4	122	140

Thermogravimetric analysis (TGA) of the hazelnut, olive, pumpkinseed and sunflower oil networks (with the aromatic bisTAD crosslinker) revealed that all products exhibited similar thermal degradation behaviour. All networks showed 5% weight loss around 275 °C while a char yield around 12% could be detected. In a final experiment, the versatility of this system was demonstrated by producing olive oil-based networks on a larger scale. Up to 10 grams of crosslinked polymer was achieved in a single batch by mixing a solution of 5 g bisTAD in a minimal amount of acetone with the crude oil. The slow gelation of the olive oil allowed easy processing of the resulting mixture, thereby facilitating the production of stiff polymeric foils and irregularly shaped materials (see Figure V.12).

Figure V.12: Plant foil obtained by crosslinking olive oil at ambient conditions with a bisfunctional TAD molecule (**9**).

V.5 Conclusions and perspectives

The unique reactivity of TAD makes it possible to readily expand the range of its complementary partners to biobased building blocks. Double bonds are present throughout natural components, one example being oils and fats of vegetable and animal origin. This chapter describes the additive-free functionalization and crosslinking strategy for crude plant oils.

First, an extensive low MW model study was performed, in which the most common fatty acids (both mono- and polyunsaturated) were treated with monofunctional TAD molecules. It was shown that the reactions went to full conversion, satisfying *click* criteria under additive-free, room temperature and ambient atmosphere conditions. These equimolar functionalization reactions can be readily monitored by NMR and MS analysis, further facilitated by the disappearance of the characteristic red colour of the TAD molecule. As a result of the nature of the implemented Alder-*ene* reaction, the fatty acid unsaturations remain present in the modified lipid tails, and can even be used in a second (slower) modification (or crosslinking). Furthermore, a selective hydrogenation of these adducts was demonstrated, leading to a saturated end product. Secondly, crude (commercially available) plant oils were crosslinked with bisTAD-molecules. Gelation occurred within minutes, while the overall reaction progress was visually monitored. By studying the gelation and thermal properties, a general trend in plant oil reactivity could

be perceived, showing that a higher percentage of polyunsaturation resulted in a shorter gelation time and a higher T_g of the obtained plant oil network. Furthermore, by simply varying the amount of crosslinker or the structure thereof, a range of different material properties, with a tunable range in T_g values, could be obtained starting from the same plant oils.

This chapter shows the great potential of the use of triazolinediones in combination with natural components. The selective reactivity for internal alkenes over terminal alkenes, makes the TAD-based *click* chemistry a quite interesting and complementary tool for plant oils in comparison to other functionalization reactions. Besides plant oils, there remains a great availability of other biobased products containing double bonds to be explored (e.g. terpenes). Since this work is outside the scope of this doctoral thesis, an additional PhD project, within our own research group, was started to investigate this in further detail.

V.6 Experimental section

V.6.1 Materials

Aluminium oxide, ethyl carbazate (97%), hydrogen chloride–ethanol solution (1.25 M), isophorone diisocyanate (98%), 4,4'-methylenebis-phenylene diisocyanate, methyl linoleate (99% (GC)), methyl linolenate (99% (GC)), oleyl alcohol (85%), palladium on carbon (5%), phenyl isocyanate, potassium hydroxide (reagent grade, 90%, flakes), soybean oil, 1-thioglycerol (99%), 10-undecenoic acid and all solvents were purchased from Aldrich and used as such. Hydrochloric acid (36%) from Chem-Lab. Castor oil was purchased from a local pharmacy (SA Aca Pharma NV) and other plant oils (pumpkinseed, sunflower, hazelnut, colza, corn and groundnut oils) were purchased in a local supermarket. All products were used without any pre-treatment or purification.

V.6.2 Charachterization

Nuclear Magnetic Resonance (NMR) ^{1}H-spectra were recorded with a Bruker Avance 300 (300 MHz) FT-NMR spectrometer in $CDCl_3$ (Eurisotop) or DMSO-d_6 solution at room temperature. Chemical shifts are presented in parts per million (δ) relative to $CHCl_3$ (7.26 ppm for ^{1}H-NMR) and DMSO (2.50 ppm for ^{1}H-NMR) as an internal standard. The resonance multiplicities are descried as [br. (broad)] s (singlet), d (doublet), t (triplet), q (quadruplet), quin (quintuplet), sext (sextuplet) or m (multiplet).

Liquid Chromatography - Mass Spectrometry (LC-MS) analyses were performed on an Agilent Technologies 1100 series LC/MSD system with a diode array detector (DAD) and a single quad MS. Analytical reversed phase HPLC-analyses were performed with a Phenomex Luna C18 (2) column (5 μm, 250 mm $\times$ 4.6 mm) and a solvent gradient (0-100 % acetonitrile in H_2O in 15 min). The eluted compounds were analyzed via UV detection (214 nm).

Differential Scanning Calorimetry (DSC) thermograms were recorded using a TA Instruments Q2000 DSC with autosampler option and Refrigerated Cooling System (RCS). Nitrogen gas was used as purge gas. The samples were studied in TAi Tzero Hermetic aluminium sample pans and at a scan rate of 10 K min^{-1}.

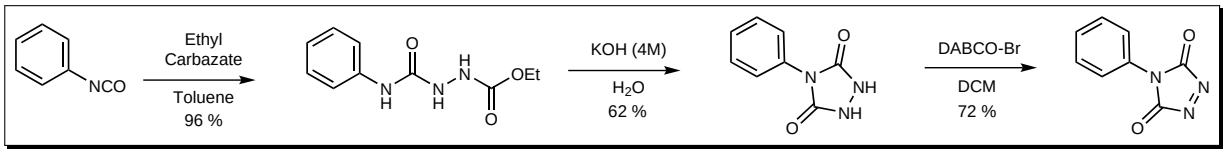
Thermogravimetric analysis (TGA) was performed using a Mettler-Toledo TGA/SDTA 851e equipment. Samples (5 to 10 mg) were heated in a nitrogen atmosphere with a heating rate of 10 K min-1 going from 25 °C to 600 °C. For the analysis of the thermograms, the STARe software of Mettler-Toledo was used.

V.6.3 Synthesis

The synthesis of the following compounds can be found in previous chapters:

- Urazole initiator for Cu(0) mediated polymerization (**56**) - see III.8.3.9
- 4,4'-(4,4'-diphenylmethylene)-bis-(1,2,4-triazoline-3,5-dione) (**9**) - see IV.7.3.2

V.6.3.1 4-phenyl-1,2,4-triazoline-3,5-dione (PhTAD)



A mixture of ethyl carbazate (10 g, 96.1 mmol, 1 equivalent) and toluene (105 mL) was placed in a three neck flask (250 mL) and cooled in an ice bath. The flask was equipped with an addition funnel, containing phenyl isocyanate (10.44 mL, 96.1 mmol, 1 equivalent), a mechanical stirrer and a bulb condenser. The mixture was put under inert atmosphere and the isocyanate was added slowly under vigorous stirring. After addition the mixture was stirred at room temperature for two hours, followed by two hours at 90 °C. After cooling down the reaction to room temperature, 4-phenyl-1-(ethoxycarbonyl) semicarbazide was filtered off and washed with toluene (96%).

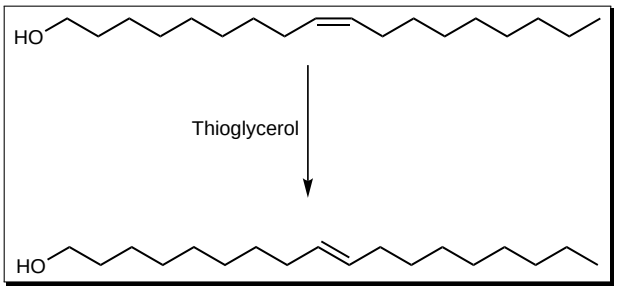
Then in a 50 mL flask, the obtained 4-butyl-1-(ethoxycarbonyl) semicarbazide (12.2 g, 60.0 mmol) was dissolved in an aqueous potassium hydroxide solution (30 mL, 4 M) under inert atmosphere. This mixture was refluxed for 1.5 hour (100 °C), warm filtrated, cooled down to room temperature and acidified until pH 1 by adding of hydrochloric acid (37%). This mixture was cooled down to room temperature to yield a white powder (4-phenyl-1,2,4-triazolidine-3,5-dione) that was filtered off (95%).

In a last step, a mixture of the just obtained 4-phenyl-1,2,4-triazolidine-3,5-dione (1 g, 5.64 mmol, 1 equivalent), DABCO-Br (2 g, 1.27 mmol, 0.2 equivalent) and dichloro-

methane (30 mL) was put in a flask (100 mL) under inert atmosphere, and stirred for two hours at room temperature. The reaction mixture was filtrated, the residue washed with dichloromethane (2 x 30 mL) and the filtrate was concentrated *in vacuo* to obtain 4-phenyl-1,2,4-triazoline-3,5-dione (PhTAD) as dark red crystals (92%).[i]

^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) = 7.45-7,60 (m, 5 H, Ar$-H$).

V.6.3.2 Elaidyl alcohol



Oleyl alcohol (1 equivalent) and thioglycerol (1 equivalent) were mixed in a flask and let to stir at room temperature after degassing (three times vacuum and nitrogen flush) overnight. The product was purified via recrystallization from methanol three times. Only a small amount of product was isolated in order to obtain highly pure product for the model reactions (yields < 50%).

^{1}H-NMR (300 MHz, DMSO$-\mathbf{d_6}$): δ (ppm) = 0.88 (t, 3 H, CH_3), 1.16-1,42 (m, 18 H, aliphatic), 1.49-1,64 (m, 2 H, aliphatic), 1.89-2,05 (m, 4 H, allylic), 3.64 (t, 2 H, HO$-$CH_2), 5.30-5,46 (m, 2 H, vinylic).

V.6.3.3 Methyl ricinoleate (77)

Castor oil was refluxed in excess of methanol and catalytic amount of sulphuric acid for two hours. The excess methanol was removed via rotavap. The residue was flushed through basic activated aluminium oxide with hexane. The product was purified with column chromatography (hexane and hexane:ethyl acetate = 9:1) to obtain a pale yellow oil (65%).

[i]The temperature of the cooling bath should not exceed 50 °C due to the volatility of the obtained compound.

^{1}H-NMR (300 MHz, DMSO$-$d$_6$): δ (ppm) = 0.86 (t, 3 H, CH_3), 1.12-1,52 (m, 18 H, aliphatic), 1.52-1,71 (m, 2 H, aliphatic), 1.94-2,09 (m, 2 H, allylic), 2.19 (t, 2 H, =CH–CH_2–CH(OH)–), 2.28 (t, 2 H, –CH_2–COOCH$_3$), 3.52-3,73 (m, 4 H, methylester- and=CH$-$CH$_2-$CH(OH)), 5.22-5,67 (m, 2 H, vinylic).

V.6.3.4 Model reactions

Model studies were performed by dissolving one equivalent of fatty acid in $CDCl_3$ (400 μL). To this, a solution of one equivalent of TAD in $CDCl_3$ (500 μL) was added and stirred until the colour faded. When necessary, a second equivalent of TAD in $CDCl_3$ (500 μL) was added and stirred at room temperature. The final products were analysed by NMR and MS without any purification.

- **1:1 adduct of oleyl alcohol and PhTAD (81)**

  ^{1}H-NMR (300 MHz**, CDCl$_3$):** δ (ppm) = 0.82 (t, 3 H, CH_3), 1.22 (band, 20 H, CH_2), 1.46 (band, 4 H, CH_2), 1.65 (m, 2 H, CH_2-CH$-$N), 1.98 (br.d, 2 H, HO$-$CH$_2-$CH_2), 3.65 (m, 2 H, HO$-$CH_2), 4.53 (q, 1 H, C$H-$N), 5.36 (m, 1 H, CH=CH), 5.71 (m, 1 H, CH=CH), 7.36 (b, 5 H, ArH).

- **1:2 adduct of oleyl alcohol and PhTAD (82)**

  ^{1}H-NMR (300 MHz**, CDCl$_3$):** δ (ppm) = 0.81 (t, 3 H, CH_3), 1.22 (band, 16 H, CH_2), 1.48 (band, 4 H, CH_2), 1.61 (m, 2 H, CH_2), 1.97 (br.d, 2 H, HO$-$CH$_2-$CH_2), 3.56 (m, 2 H, HO$-$CH_2), 4.10 (m, 1 H, CH$_2-$C$H-$N), 4.52 (q, 1 H, CH$-$C$H-$N), 5.39 (m, 1 H, CH=CH), 5.70 (m, 1 H, CH=CH), 7.37 (b, 10 H, ArH).

- **1:1 adduct of elaidyl alcohol and PhTAD**

  ^{1}H-NMR (300 MHz**, CDCl$_3$):** δ (ppm) = 0.88 (t, 3 H, CH_3), 1.29 (band, 20 H, CH_2), 1.48 (band, 2 H, CH_2), 1.61 (m, 2 H, CH_2), 2.04 (br.d, 2 H, HO$-$CH$_2-$CH_2), 3.61 (m, 2 H, HO$-$CH_2), 4.52 (q, 1 H, CH$-$C$H-$N), 5.39 (m, 1 H, CH=CH), 5.70 (m, 1 H, CH=CH), 7.37 (b, 10 H, ArH).

- **1:1 adduct of methyl ricinoleate and PhTAD**

  ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) = 0.88 (t, 3 H, CH_3−CH_2), 1.30 (band, 19 H, CH_2), 1.51 (m, 2 H, CH_2), 1.62 (m, 2 H, CH_2), 2.30 (t, 2 H, CH_3−C−CH_2), 3.67 (s, 3 H, CH_3−C), 4.98 (q, 1 H, CH−CH−N), 5.49 (m, 1 H, CH=CH), 5.70 (m, 1 H, CH=CH), 7.37 (b, 5 H, ArH).

- **1:1 adduct of 10-undecenoic acid and PhTAD**

  A mixture of products is obtained. The relevant new signals (newly formed double bonds + aryl) are:

  ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) = 5.45 (m, CH), 5.83 (m, CH), 7.36 (m, 5 H, ArH).

- **1:1 adduct of methyl linoleate and PhTAD (83)**

  A mixture of regioisomers is obtained. The relevant new signals (newly formed double bonds + aryl) are:

  ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) = 5.50-6,2 (b, CH), 7.36 (m, 5 H, ArH).

- **1:1 adduct of methyl linolenate and PhTAD (83)**

  A mixture of regioisomers is obtained. The relevant new signals (newly formed double bonds + aryl) are:

  ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) = 5.90-6,2 (b, CH), 7.36 (m, 5 H, ArH).

- **1:1 adduct of oleyl alcohol and ATRP-TAD (85)**

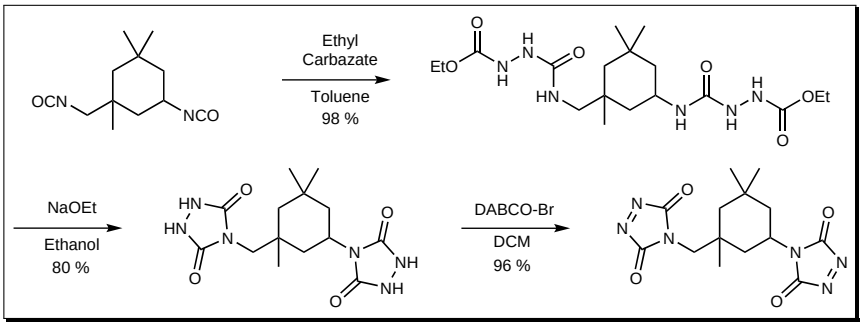
  ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) = 0.80 (t, 3 H, CH_3−CH_2), 1.20 (band, 22 H, CH_2), 1.51 (m, 2 H, CH_2), 1.62 (m, 2 H, CH_2), 2.00 (s, 6 H, CH_3−CBr), 3.67 (t, 2 H, HO−CH_2), 4.51 (q, 1 H, CH−CH−N), 5.35 (m, 1 H, CH=CH), 5.70 (m, 1 H, CH=CH), 7.37 (d, 2 H, ArH), 7.57 (d, 2 H, ArH), 8.36 (s, 1 H, Ar−NH).

V.6.3.5 Hydrogenation of adduct 81 to obtain 84

The adduct **81** from the model study was dissolved in methanol (10 wt%) and a catalytic amount of palladium on carbon (5%) was added. The mixture was stirred under hydrogen atmosphere overnight at room temperature. Finally, the solution was filtered over celite and concentrated *in vacuo* to obtain the hydrogenated product **84**.

^{1}H-NMR (300 MHz**, $CDCl_3$):** δ (ppm) = 0.82 (t, 3 H, CH_3), 1.22 (band, 24 H, CH_2), 1.65 (band, 6 H, CH_2−CH−N + HO−CH$_2$−CH_2), 3.51 (m, 2 H, HO−CH_2), 4.10 (q, 1 H, CH−N), 7.36 (b, 5 H, ArH).

V.6.3.6 IPDI-bis-(1,2,4-triazoline-3,5-dione) (87)



A mixture of ethyl carbazate (40 g, 0.384 mol, 2 equivalents) and toluene (300 mL) was placed in a three neck flask (1 L) and cooled down in an ice bath. The flask was equipped with an addition funnel, containing isophorone diisocyanate (IPDI, 39.3 g, 0.192 mol, 1 equivalent) diluted in toluene (200 mL), a mechanical stirrer and a bulb condenser. The mixture was put under inert atmosphere and the isocyanate was added slowly under vigorous stirring. After addition the mixture was stirred at room temperature for two hours, followed by two hours at 90 °C. After cooling down the reaction to room temperature, IPDI-bis-(carbethoxysemicarbazide) was filtered off and washed with toluene (75.9 g – 98%).

Then in a 100 mL flask, the obtained IPDI-bis-(carbethoxysemicarbazide) (2 g, 4.6 mmol) was dissolved in an sodium ethoxide solution (50 mL, 4 wt%) under inert atmosphere. This mixture was refluxed for 24 hours (85 °C), warm filtrated, cooled down to room temperature and acidified until pH 1 by adding of hydrogen chloride–ethanol solution (1.25 M). This mixture was cooled down to room temperature and a white powder was

filtered off. By removing the solvent *in vacuo* the desired product (IPDI-bis-urazole) was obtained as a fine powder (1.25 g – 80%).

In a last step, a mixture of the just obtained IPDI-bis-urazole (2 g, 5.64 mmol, 1 equivalent), DABCO-Br (5 g, 3.18 mmol, 0.58 equivalent) and dichloromethane (30 mL) was put in a flask (100 mL) under inert atmosphere, and stirred for five hours at room temperature. The reaction mixture was filtrated, the residue washed with dichloromethane (2 x 30 mL) and the filtrate was concentrated *in vacuo* to obtain IPDIbisTAD as dark red crystals (1.81 g – 96%).[ii]

^{1}H-NMR (300 MHz, DMSO$-$d$_6$): δ (ppm) = 0.87 (br.s, 3 H, C$-$CH_3), 0.98 (br.s, 2 x 3 H, C$-$CH_3), 1.03-2,15 (m, 6 H, C–CH_2–C), 2.82 (br.d, 2 H, N$-$CH_2), 3.87 (br.s, 1 H, N$-$CH).

V.6.3.7 Plant oil network

For the synthesis of the networks, 100 mg (unless otherwise mentioned) of plant oil was dissolved in acetone (200 µL) and to this 4–4'-methylenediphenyldi-1,2,4-triazoline-3,5-dione (100 mg (unless otherwise mentioned)) or IPDI-TAD (100 mg (unless otherwise mentioned)) in acetone (500 μL) was added and stirred until gelation occurred. The gel was left at room temperature until the colour faded (in the order of minutes). The obtained networks were dried overnight in a vacuum oven at 40 °C prior to the thermal analysis.

[ii] The temperature of the cooling bath should not exceed 50 °C due to the volatility of the obtained compound.

V.7 Bibliography

[1] P. Anastas, N. Eghbali, *Chemical Society Reviews* **2010**, *39*, 301–312.

[2] PlasticsEurope, *Plastics - The Facts 2013*, **2013**.

[3] U. Biermann, U. Bornscheuer, M. A. R. Meier, J. O. Metzger, H. J. Schäfer, *Angewandte Chemie International Edition* **2011**, *50*, 3854–3871.

[4] L. Montero de Espinosa, M. A. R. Meier, *European Polymer Journal* **2011**, *47*, 837–852.

[5] N. Kolb, M. Winkler, C. Syldatk, M. A. R. Meier, *European Polymer Journal* **2014**, *51*, 159–166.

[6] M. Firdaus, M. A. R. Meier, *European Polymer Journal* **2013**, *49*, 156–166.

[7] H. Baumann, M. Bühler, H. Fochem, F. Hirsinger, H. Zoebelein, J. Falbe, *Angewandte Chemie International Edition* **1988**, *27*, 41–62.

[8] K. Visek in *Kirk-Othmer Encyclopedia of Chemical Technology, Fatty Amines*, *Vol. 2*, John Wiley and Sons, Inc., **2000**, book section 1, pp. 519–537.

[9] M. R. Infante, L. Pérez, M. C. Morán, R. Pons, M. Mitjans, M. P. Vinardell, M. T. Garcia, A. Pinazo, *European Journal of Lipid Science and Technology* **2010**, *112*, 110–121.

[10] K. Kosswig in *Surfactants*, Wiley-VCH Verlag GmbH and Co. KGaA, **2000**, book section 1, pp. 431–505.

[11] H. Wagner, R. Luther, T. Mang, *Applied Catalysis A: General* **2001**, *221*, 429–442.

[12] M. P. Schneider, *Journal of the Science of Food and Agriculture* **2006**, *86*, 1769–1780.

[13] F. N. Jones, *Ullmann's Encyclopedia of Industrial Chemistry, Alkyd Resins*, **2003**.

[14] J. A. Melero, J. Iglesias, G. Morales, *Green Chemistry* **2009**, *11*, 1285–1308.

[15] P. Gallezot in *Metal catalysis in the conversion of biosustainable resources.* (Ed.: H. J. A. J. Renouprez), Springer, Berlin, **1997**, pp. 201–221.

[16] K. Weissermel, H. Arpe, *Industrial Organic Chemistry*, Wiley-VCH, Weinheim, **1998**.

[17] A. Guo, D. Demydov, W. Zhang, Z. Petrovic, *Journal of Polymers and the Environment* **2002**, *10*, 49–52.

[18] D. Quinzler, S. Mecking, *Angewandte Chemie International Edition* **2010**, *49*, 4306–4308.

[19] O. Turunc, M. A. R. Meier, *Green Chemistry* **2011**, *13*, 314–320.

[20] T. T. Ho, T. Jacobs, M. R. Meier, *ChemSusChem* **2009**, *2*, 749–754.

[21] O. Turunc, M. Firdaus, G. Klein, M. A. R. Meier, *Green Chemistry* **2012**, *14*, 2577–2583.

[22] J. Hong, Q. Luo, X. Wan, Z. S. Petrović, B. K. Shah, *Biomacromolecules* **2012**, *13*, 261–266.

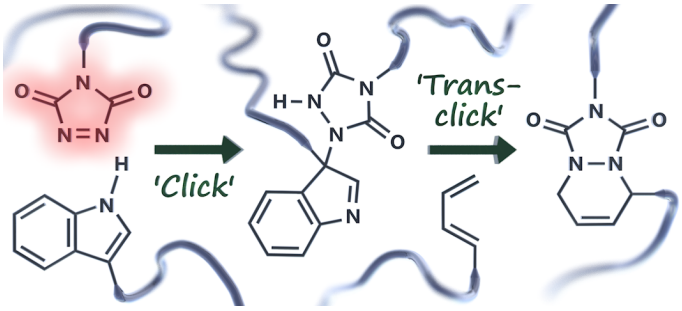
[23] C. Vilela, A. J. D. Silvestre, A. Gandini, *Journal of Polymer Science Part A: Polymer Chemistry* **2013**, *51*, 2260–2270.

[24] U. Shah, J. Lay, JacksonO, A. Proctor, *Journal of the American Oil Chemists' Society* **2013**, *90*, 155–158.

[25] A. Biswas, H. N. Cheng, S. Kim, Z. Liu, *Journal of the American Oil Chemists' Society* **2014**, *91*, 125–131.

[26] U. Biermann, W. Butte, R. Koch, P. A. Fokou, O. Türünç, M. A. R. Meier, J. O. Metzger, *Chemistry – A European Journal* **2012**, *18*, 8201–8207.

[27] F. Carey, R. J. Sundberg, *Advanced Organic Chemistry - Part B: Reactions and Synthesis*, 5th ed., **2007**.



Abstract:

Combining the modular, covalently bonded nature of *click*-chemistry linkages with an ability to reverse these linkages and reuse the constituent reactants in another *click* reaction is a feature that is not found in most *click* reactions. This chapter will introduce the unique reactivity of triazolinediones with indoles that shows that these molecules readily undergo a *click-like* reaction, and that the reverse reaction can also be induced at elevated temperatures. The resulting triazolinedione can be reacted with a different reaction partner, either reversibly or irreversibly depending on its exact nature. This reaction was shown in extensive model studies and supported by theoretical calculations. In addition, this so-called '*transclick*' reaction was used to introduce thermoreversible links into both blockcopolymers and networks. In the latter case this led to dynamic polymer-network healing, reshaping and recycling.

Reference:

Billiet S., De Bruycker K., Driessen F., Goossens H., Van Speybrouck V., Winne J.M., Du Prez F.E., *Nature Chemistry* **2014**, *6*, 815-821.

Chapter VI

Development of reversible TAD *click* and *transclick* reactions for polymer chemistry

VI.1 Introduction

As stated in chapter IV, the concept of *click* chemistry was introduced by Sharpless and co-workers[1] and the involved reactions were rapidly adopted in many research disciplines, even outside the chemical sciences.[2,3] Although this concept is almost 15 years old and many alternative systems for the archetypal *copper-catalysed azide–alkyne cycloaddition*, each with their advantages and disadvantages, have been suggested in the last years[3–5], the quest for a truly versatile system still continues. In the ideal case, such a system, besides having all the characteristics associated with the *click* concept, should also offer '*tunability*'. This tunable behaviour is crucial for many applications and operates at two levels (see Figure VI.1). The first level, *kinetic tunability*, provides control over the kinetic behaviour of the forward chemical reaction and depends on the nature of the chosen reaction partner. The second level depends more on the nature of the formed bond. In this way, if desired, weaker chemical bonds can be chosen and the original building blocks are more readily reformed upon heating – *thermodynamic tunability*. In the case of polymer chemistry applications, for example, dynamic or reversible covalent bonds are highly desired nowadays as they can be used to elicit unique material properties, such as self-healing, recycling and network malleability.

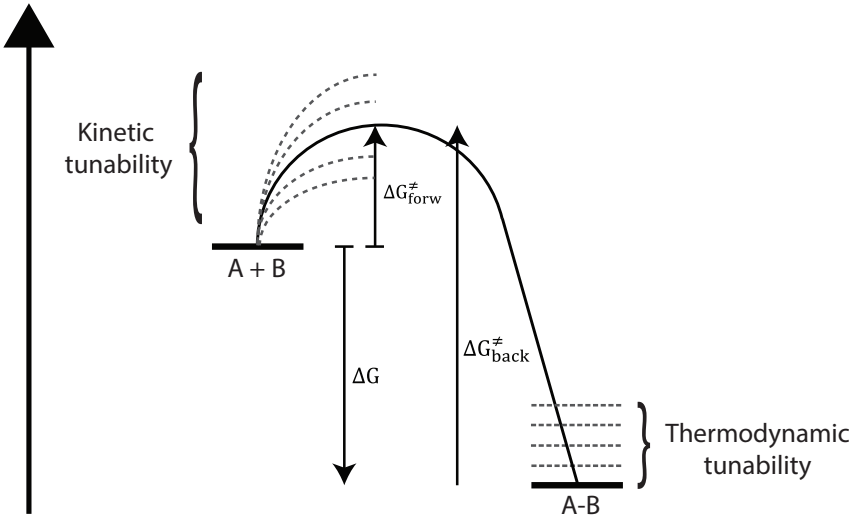


Figure VI.1: Tuning of a *click* reaction on two levels, leading to kinetic and/or thermodynamic tunability ($\Delta G + \Delta G^{\neq}_{forw} = \Delta G^{\neq}_{back}$).

Up till now, no general *click*-chemistry system has been reported that can be tuned for either reversible or completely irreversible covalent linking within useful temperature ranges. In previous chapters, the *click*-chemistry platform based on the reactivity of a 1,2,4-triazoline-3,5-dione (TAD) component was introduced and it was shown that *kinetic tunability* was possible by selecting the correct complementary partner (see section III.4). Here, the goal is to combine the catalyst-free, room-temperature reactivity of TADs with the possibility of a reversible bond formation (see Figure VI.2). Therefore in this chapter a reversible TAD-based chemistry is introduced, more specifically the reversible Alder-*ene* reaction between TAD and indoles has been studied. First, the *click* behaviour of the TAD-indole reaction will be studied on model compounds to ensure a well-behaved (forward) *click* reaction. Following this, the formed adducts will be subjected to experiments that probe the reversibility of this *click* reaction, in order to determine the necessary temperature trigger to (partially) release the starting compounds from their adducts.

The experiments in this section (*vide infra*) have led to the introduction of a new *click* chemistry concept, more specifically the '*transclick*' concept that will be discussed in detail. In line with the irreversible pathways, discussed in chapter IV, the reversible process will also be submitted to theoretical rationalization to develop a theoretical model. To bring this to a macromolecular level, block copolymers and networks are prepared and tested on their thermo-reversible behaviour. For this, a variety of dynamic materials are synthesized and fully characterized.

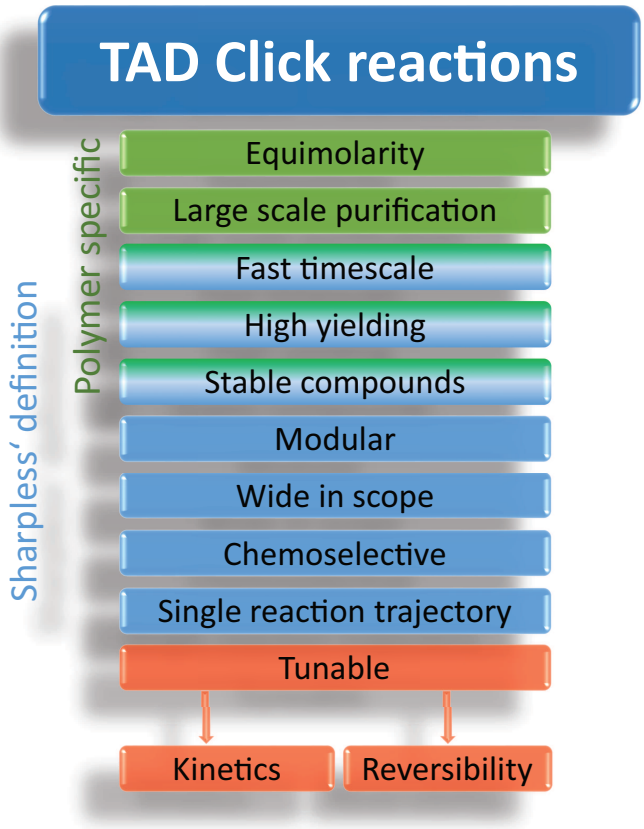


Figure VI.2: Characteristics of TAD *click* reactions (blue: originally defined by Sharpless; green and blue–green: adapted requirement related to synthetic polymer chemistry and red: additional requirements for tunable behaviour).

VI.2 Model study of dynamic TAD-reactions using low MW substrates

In 2003, Baran *et al.* found that indole compounds were regenerated from their *ene*-type adducts with 4-methyl-1,2,4-triazoline-3,5-dione (MeTAD) by simply heating the neat compounds *in vacuo* at 100 −150 °C, which removes the volatile MeTAD (see section II.5.4).[6] This remarkably efficient reversible TAD-based chemistry, which was described as a protecting-group strategy for indole functionalization, is much more interesting than the other reversible systems (e.g. adamantyl-based - see section II.3.4). Indeed, indoles are basic heterocyclic scaffolds that can be easily prepared in high yields from inexpensive starting materials via various straightforward routes. Furthermore, although indoles are mostly used as fine chemicals in the pharmaceutical and fragrance industry, they are also applied as bulk additives in high-volume polymers, such as polyvinyl chloride (PVC).[7] From the work of Baran *et al.*, it became clear that some specific structural characteristics

for an indole building block would assure a reversible adduct formation at a reasonable temperature. As can be seen in Figure VI.3, the *ene*-adduct will be formed at 0 °C while the required temperature for the retro-*ene* reaction is dependent on the substitution pattern of the indole component. When the 2,3-π-bond possesses allylic protons, the *ene* reaction will form an enamine, which is stable up to 250 −280 °C. If however, there are no allylic hydrogens at the *C2*-position (by simply introducing a quaternary carbon), the *ene*-reaction will result in an imine, which shows a remarkably lower retro-*ene*-temperature (100 −150 °C).

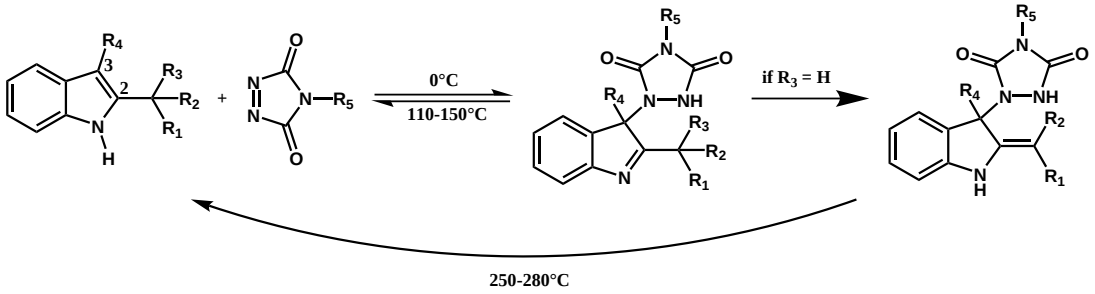


Figure VI.3: Reaction between ^1H-indole and TAD, showing the importance of the 2-position to avoid a higher temperature to obtain reversibility (see also section II.5.4).

The easily synthesized (prepared on 50 gram scale) and commercially available 2-*tert*-butylindole (**88**) was identified as a suitable starting material, which gave access to a wide range of functionalized indoles in a single step through a straightforward reductive alkylation with various aldehydes.

VI.2.1 Synthesis of indole substrates and their *click-like ene* reactions with TAD

Similar as in the case of the 'normal' TAD Diels-Alder (DA) and Alder-*ene* (AE) reactions, the (possible) *click* character has to be verified. Therefore, the TAD-indole AE reaction was checked with a series of different indole compounds in combination with an equimolar amount of 4-butyl-1,2,4-triazoline-3,5-dione (BuTAD, **31**). The involved indole model compounds are: the non-functional 2-*t*-butyl-3-*iso*pentylindole (**34**, to check for possible side reactions), an alcohol containing indole (**89**, as precursor for indole functionalization) and an indole-diol (**90**, which will be used as a monomer later on) (see Figure VI.4a). The synthetic procedure to make those compounds can be found in the experimental section. All model reactions were performed under equimolar conditions of indole and

TAD in DMSO-d_6 and checked via ^{1}H-NMR. All of them went to completion in less than one minute and led to a single reaction product (see Figure VI.4b), also in line with the orthogonality study discussed in chapter III (see section III.2).

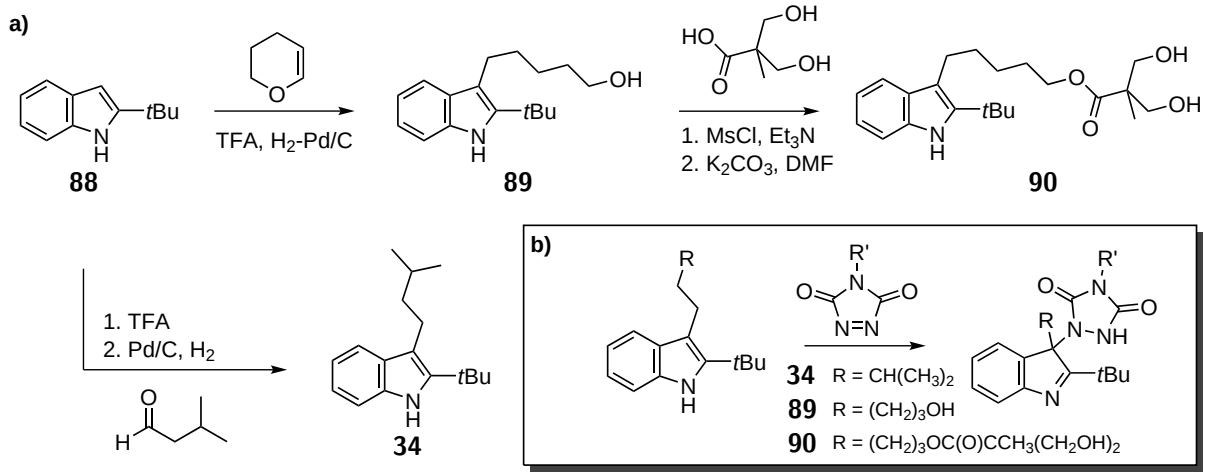


Figure VI.4: a) Synthesized indole components, based on the commercial 2-*t*Butyl-1*H*-Indole (**88**), that are used for the model study and b) their *click* reaction with TAD.

For the TAD-indole reactions, again the remaining *click* characteristics (see Figure VI.2) had to be checked. The synthesis of indoles can be conducted on large-scale (100 g scale reactions can be performed), without the need of difficult purification methods such as chromatography. The same can be said for TAD components, as was already discussed in the previous chapter. In section III.4 we have seen that the rate constants for TAD-indole reactions are high, although a bit lower than all carbon dienes and tetrasubstituted enes. Thus, it can be concluded that TAD-indole AE reactions can also be classified as a *click* reaction.

VI.2.2 Investigation of the dynamic nature of the TAD-indole *click* reaction

In order to check the reversible nature of the TAD-indole *click* reaction, the TAD-indole adduct (**91**), that was obtained from the reaction between BuTAD (**31**) and 2-*t*-butyl-3-*iso*pentylindole (**34**), was submitted to a thermal treatment (120 °C in DMSO-d_6 for 30 minutes, similar as for dienes - see chapter IV). The solution contained only the TAD-indole adduct (no dienes, indoles or other additives were added), so it was expected that this experiment would be able to confirm the reversible behaviour. However, no traces of

the original starting components were observed (both on LC-MS and ^{1}H-NMR). This leads to the conclusion that the equilibrium is still very much in favour of the adduct, while reports in literature suggest significant reversibility at this temperature.[6] Although ^{1}H-NMR seems like the perfect method to study AE reactions between BuTAD and indole compounds - by performing high temperature NMR experiments - we were unable to observe free TAD compounds at elevated temperatures, also confirmed by the absence of red colour in heated solutions. This made it necessary to look for an indirect method to study the dynamic equilibrium.

A practical solution was found in adding a alternative partner for TAD, which makes irreversible connections with TADs. Since it was shown in chapter IV, that *trans*,*trans*-2,4-hexadiene-1-ol (HDEO, **32**) – and other dienes – react very fast with TAD, this seemed an appropriate substrate for these model studies. The formed DA-adduct was already confirmed to be stable to temperatures above 200 °C. Thus, when the solution is heated (and the equilibrium is pushed relatively towards the starting products), the liberated TAD molecules can, in this way, react via two pathways: one leading to the original reversible product (TAD-indole) and the other (much faster pathway) to an irreversible product (TAD DA-adduct) - see Figure VI.5. The amount of formed DA-adduct, in a certain time frame, will therefore be an indication of the total amount of TAD molecules that were released from the original adduct, in that same time frame.

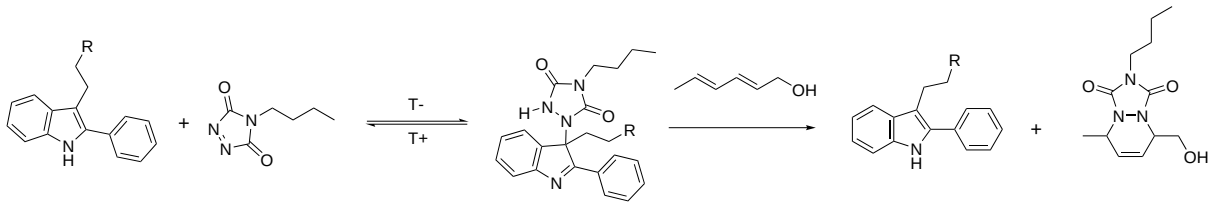


Figure VI.5: General scheme of the model study between BuTAD (**31**) and one of the indole model components. At room temperature the *ene*-adduct is quantitatively formed while a retro-*ene* reaction occurs at elevated temperature. By the addition of a diene, an indirect proof of the reversiblity can be visualized.

Since the model indole components studied in these reversibility experiments (**34**, **89** and **92**) all have similar chemical environments around the indole core, the resulting reactivity profiles were almost identical. For this reason only one of these experiments will be discussed in detail. Figure VI.6a shows all components that can be present in the model study, namely the indole-diol (**90**), the *ene* adduct (**91**) of BuTAD and the indole-

diol, HDEO (**32**) and the DA-adduct (**33**) of HDEO and BuTAD. In a first step, a stock solution of indole-diol (**90**) and BuTAD (**31**) in DMSO-d_6 was prepared from which all NMR samples were collected (details see experimental part). After stirring for one minute the progress of the reaction was monitored via thin layer chromatography (TLC). BuTAD (**31**) was added in a small excess to the indole substrate (1.05 equivalent) in order to assure complete reaction of the indole, such that no trace of indole would remain present in the reaction mixture due to small imperfections in the stoichiometry in equimolar reactions. As expected, this resulted in the persistence of a pink colour in the obtained solutions. For this initial mixture, any trace of free indole would thus be the result of a retro-*ene* reaction. The NMR spectrum of the formed *ene*-adduct (**91**, see Figure VI.6b) shows on the one hand that the signal for the indole NH ***(H-1)*** is replaced by the one for the urazole NH (***H-3***). Simultaneously the protons of the methylene group (that are connected to the *C3* position of the indole) also show a significant change (signal (***H-2***) is replaced by signal (***H-4***)). A last clear difference is that the *ene*-reaction causes a change in the chemical shift of the aromatic region, which can be explained by the removal of the electron donating nitrogen lone pair. In a last step, 1.1 equivalent of HDEO (**32**) is added to the mixture (causing the pink colour to disappear immediately), in order to irreversibly 'cap' any free TAD compounds that are liberated at higher temperature. The ^{1}H-NMR spectrum (Figure VI.6b) of the stock solution shows that the presence of HDEO (**32**) does not affect the *ene*-adduct (**91**) at ambient temperature. It can be noted, however, that the small excess of BuTAD resulted in the immediate appearance of a small amount of the corresponding DA-adduct (**33**) - see signal (***H-7***) in Figure VI.6b.

According to the initial report of Baran *et al.*, it can be expected that the retro-*ene*-reaction occurs, at a significant reaction rate, somewhere between 110 and 150 °C.[6] Thus, a temperature range going from 50 to 160 °C (in steps of 10 °C) was tested in separate 15 minute heating experiments (using different solutions). The NMR samples were subjected to 15 minutes at the desired temperature before being rapidly cooled and subjected to NMR analysis. It is observed, as can be seen from Figure VI.6, that the mixture of TAD-indole *ene*-adduct (**91**) and HDEO (**32**) can be completely converted to a solution of the original indole (**90**) and DA-adduct (**33**), after heating to 140-160 °C for just 15 minutes. Thus, as expected the retro-*ene*-reaction liberates the indole (**90**) and BuTAD (that is directly transformed into the DA-adduct **33**), which was confirmed by the NMR data (see

Figure VI.6c). It can be seen that, at a temperature of 100 °C already a small amount of indole-diol (**90**) is released from the *ene*-adduct (**91** - signals ***(H-1)*** and ***(H-2)***). This process, however, proceeds with an unexpected efficiency given the highly reactive nature of TAD compounds at higher temperatures, which could give a large number of side reactions.

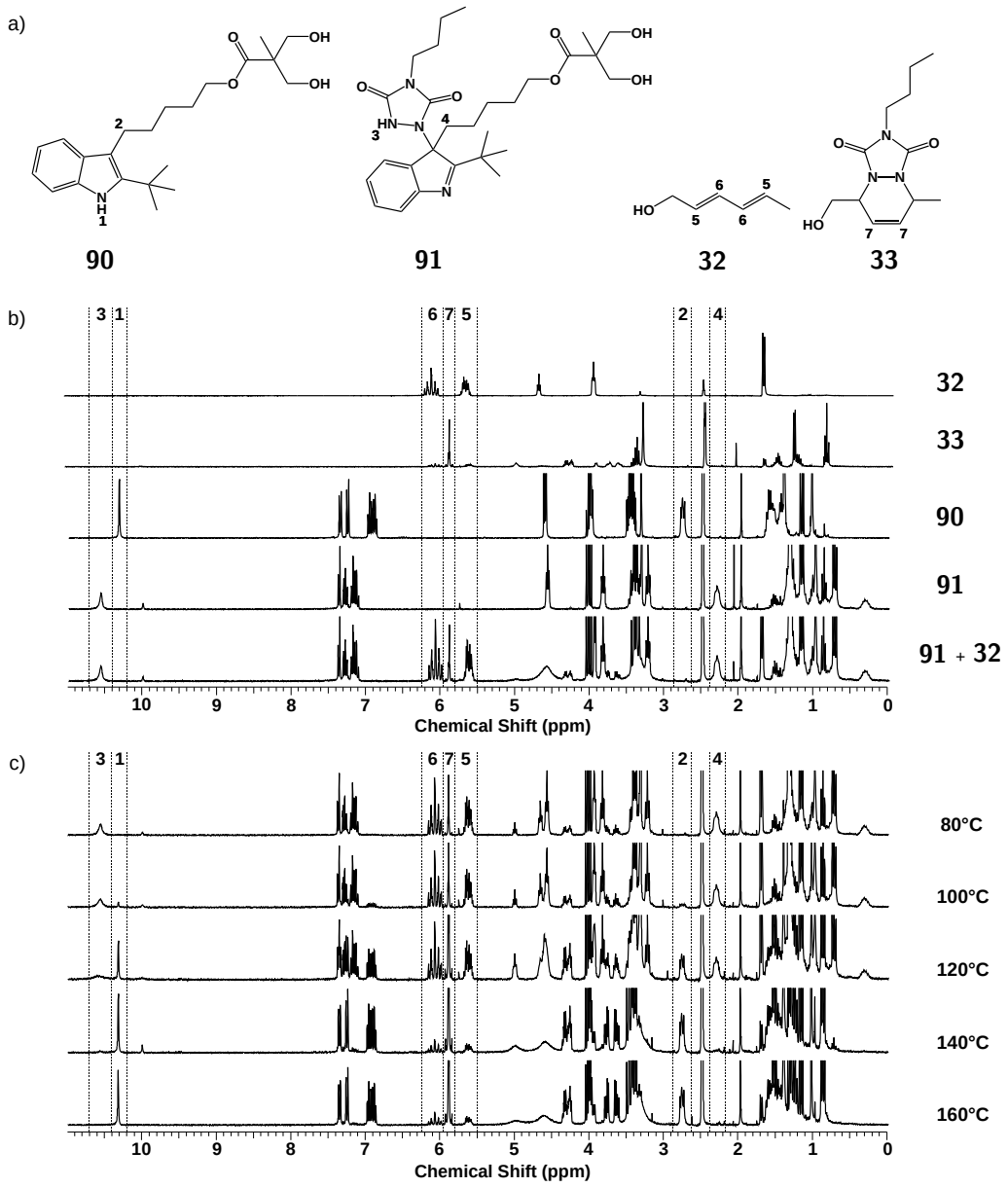


Figure VI.6: ^{1}H-NMR study to show the reversibility of the indole-TAD adduct (**91**). a) used components, b) NMR spectra of isolated compounds and mixtures and c) evolution of NMR spectra of samples (containing indole-TAD adduct (**91**) and HDEO (**32**)) that are treated for 15 minutes at the indicated temperature (and subsequently cooled down to room temperature before performing the experiment).

After heating to 120 °C for 15 minutes, both the *ene*-adduct (**91**) and the indole diol (**90**) are already present as major constituents in the solution (>25%), while the same treatment at 140 °C suffices to release all the indole diol. (see signals ***(H-3)*** and ***(H-4)***). The small amount of DA-adduct (**33**), already present in the stock solution, makes it difficult to use signal ***(7)*** to see a clear increase in the DA-adduct. However signals ***(H-5)*** and ***(H-6)*** – coming from HDEO (**32**) – show a clear decrease with increasing temperatures, which implies that the liberated BuTAD (**31**) undergoes a DA-reaction. It has to be noted that in this series of spectra no signals appear that cannot be accounted to one of the described structures in Figure VI.6a. This suggests that at lower nor higher temperatures any side reactions occur. To check this promising fact, the same study was repeated via *Liquid Chromatography – Mass Spectrometry* (LC-MS) (see Figure VI.7) (**33**). These spectra confirm that in the stock solution only the *ene*-adduct (**91**) and a small quantity of DA-adduct are present, while after heating to 160 °C only the indole diol (**90**) and the DA-adduct (**33**) can be detected.

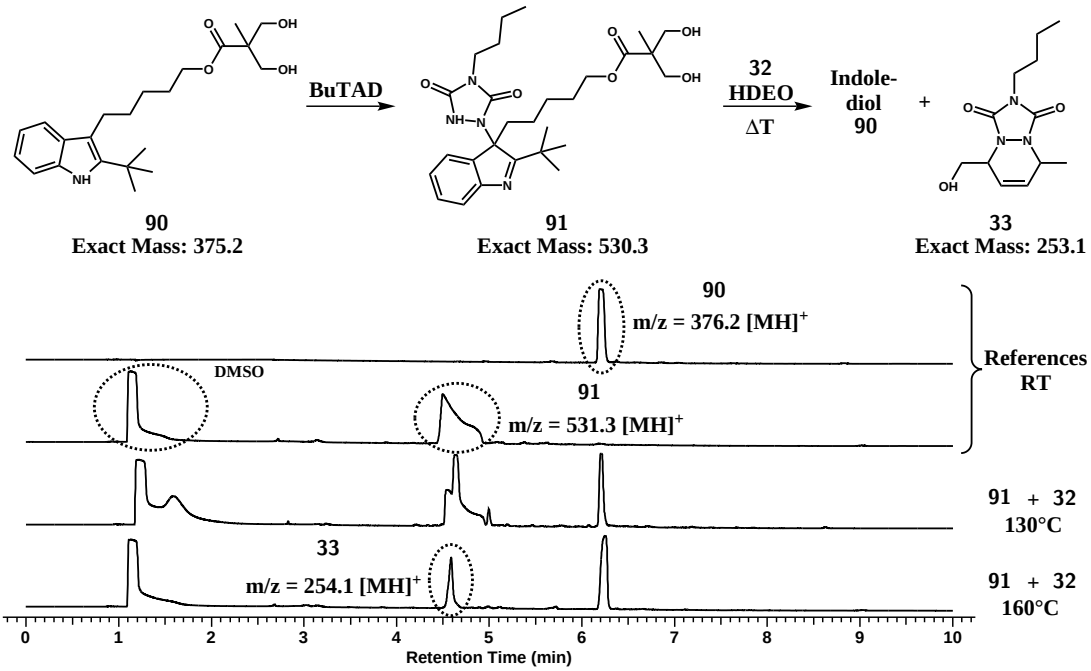


Figure VI.7: LC-MS analysis of the reversibility study (UV chromatograms with $\lambda = 214$ nm given) of the indole-diol (**90**) and BuTAD.

In conclusion, the above experiments show a remarkably efficient transfer-reaction of a TAD-group from an indole to a diene reaction partner. This (clean) *click-like* transfer of reagents from one substrate to another was not described yet for any other *click* reaction. Thus, a new term is suggested to describe these kinds of reactions. We introduced the

term '*transclick*' reaction, in analogy with well-known chemical processes such as transesterifications or transalkylations.[8] In other words, a *transclick* reaction can be defined as: *any covalent linking process that subsequently can be triggered to form a new bond with an alternative or orthogonal reaction partner, and at the same time release one of the original binding partners, in which both bond-forming steps meet the usual requirements for click reactions.*

The exchange rates in the TAD-indole *transclick* reaction can be quantified to some extent via NMR. The integration of signal **(*H-4*)** in the spectrum of the stock solution provides a measure for the total amount of *ene*-adduct that is remaining in the mixture. The fraction of released indole diol can easily be measured (integration of signal **(*H-2*)**). Figure VI.8 gives an overview of the reversibility profiles of all involved components. The fraction of released indole (which is a measure of the percentage of the *transclick* reaction) is plotted against the temperature at which the *transclick* reaction was *unclicked*. Since the retro-*ene* reaction between TAD and indole takes place over a rather large temperature range, it became a necessity – inspired by the work of Zhou *et al.*[9] – to introduce an arbitrary qualitative threshold of liberated indole (or % of *transclick*) that defines the reversibility temperature of the TAD-indole system. So a comparison of temperatures is made where, after 15 minutes of heating, 5% of the original indole component is recovered. Figure VI.8 shows that, according to this definition, below a temperature of 90 °C the exchange rate is not yet very significant (during 15 minutes of heating). Starting from this temperature the retro-*ene*-reactions becomes increasingly important. When heated at 150 °C (for 15 minutes) all of the indole component is released.

As can be seen in Figure VI.8, the exact structure of the side chain in indoles **34**, **89** and **90** appears to have no influence on the reversibility of the reaction. The differences between the fractions of free indole (% *transclick*) for the different indole components all fall within the error margin of the NMR measurement.

In summary, the results obtained on the dynamic nature of the indole-TAD *click* reactions broaden the scope of the introduced triazolinedione platform to unprecedented *click* chemistry applications. We showed a highly promising, reversible and orthogonal *click* reaction between indoles and TAD, which allows a 'programmed' *transclick* reaction of a TAD compound from one partner to another. It has to be noted that more quantitative

kinetic experiments, which would allow to determine crucial parameters such as activation energy barriers, are necessary to have a complete overview of the reversible behaviour. These will be carried out in the near future within our own research group.

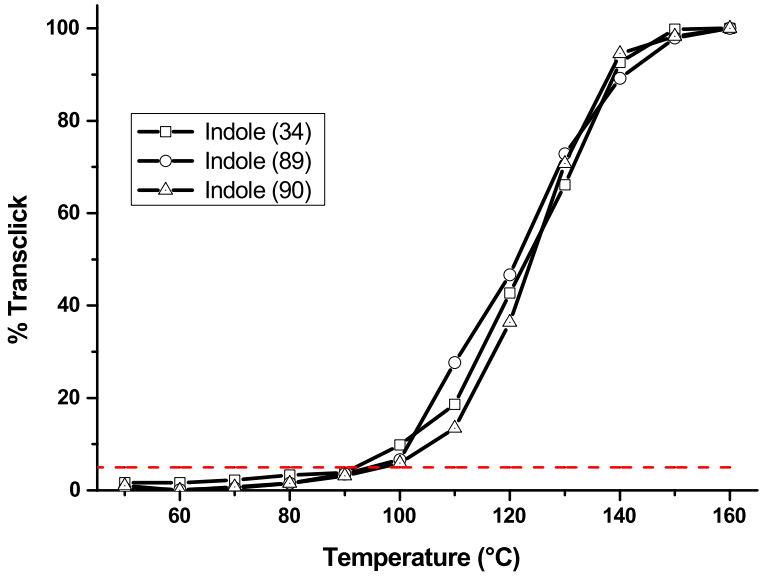


Figure VI.8: Off-line ^{1}H-NMR reversibility study for 2-*tert*-butyl-3-isopentyl-1*H*-indole (**34**), 2-*tert*-butyl-3-pentan-1-ol-1*H*-indole (**89**) and indole diol (**90**). The indole component was mixed with BuTAD in a 1:1 ratio after which one equivalent of HDEO (**32**) was added and the mixture was heated in DMSO-d_6. Every data point is the result of heating a (new) sample for 15 minutes at the indicated temperatures. The red line on the curve represents the 5% limit where upon the reversibility temperature is determined for each indole.

VI.3 Computational study: theoretical rationalization

Similar as in the case of the irreversible coupling (see section IV.3), the mechanism of this reversible pathway was, in collaboration with dr. Hannelore Goossens en prof. Veronique Van Speybroeck of the *Centre of molecular modeling* (CMM), studied by means of *density functional theory* (DFT) to better understand the factors that influence the barriers of forward and reverse reactions and to help the design of suitable polymer building blocks and materials in a later stage. As a simplified model, the *ene* reaction of BuTAD with 2-*t*-butyl-3-methylindole was used (see Figure VI.9) and it was found to follow a stepwise route via an *iminium–urazolide zwitterionic intermediate* (IZ, $\Delta G^{\neq} = 70.3\,\mathrm{kJ/mol}$). This intermediate zwitterion is readily converted into the reaction product by a simple proton

transfer.

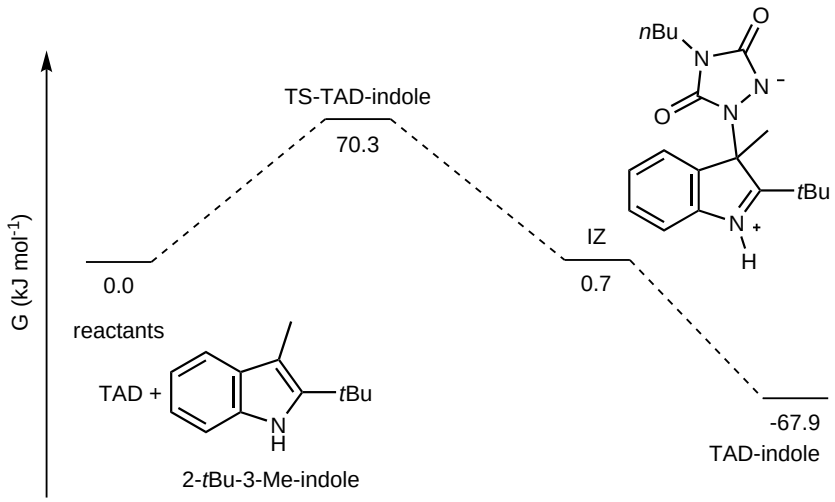


Figure VI.9: Free energy profile (kJ/mol) for the TAD-Indole reaction (PCM ($\epsilon = 8.93$) M06-2X/6-31+G(d,p), IZ stands for iminium-urazolide zwitterionic intermediate and TS for transition state).

Once this was established, in a first step the forward reaction is checked into more detail and specifically towards its *click* behaviour. As already discussed in the previous chapter, a *click* reaction should possess a large thermodynamic driving force ($\Delta G < -84\,kJ/mol$). Similar as its irreversible counterpart, the reversible TAD-Indole reaction approaches this and thus favours a single reaction product. For the reverse reaction, an energy barrier of 138.2 kJ/mol can be deduced. This remarkably lower barrier shows the possibility of the retro-reaction at lower temperatures. The reversible nature of the TAD-indole *click* reaction, related to the lower exergonicity of the forward reaction can also be readily related to the loss of aromatic resonance energy of the indole starting material.

The main message to be taken from this, is the confirmation that the forward reaction shows irreversible *click* behaviour at lower temperatures, while the relatively low retro-barrier suggests a reversible character at higher temperatures. In addition, if there would be a need of other complementary partners for TAD molecules, these calculations (reversible and irreversible pathways) will be a useful tool to predict the stability (and thus reversible character) of the formed adducts at elevated temperatures.

VI.4 Reversible polymer functionalisation and conjugation using TAD-indole *click* reactions and indole-diene *transclick* reactions

In order to test the reversible *click* and *transclick* reactions in a macromolecular context, an indole-functionalized poly(methylmethacrylate) (PMMA) sample was synthesized using an indole-containing alkyl bromide initiator (**93** - see VI.7.3.4). The polymer (indole–PMMA) was obtained via a Cu-mediated polymerization with an average molecular weight (M_n) of 8300 g/mol and a dispersity ($Đ$) of 1.32. Next, this polymer was reacted with one equivalent of the low-molecular weight BuTAD to give a clean conversion into the indole–TAD–*ene* adduct (as shown by ^{1}H NMR - see Figure VI.10 – and SEC). The resulting TAD–indole–PMMA was then dissolved in DMSO with an excess of HDEO. This mixture was heated for 30 minutes at 120 °C, which resulted in the parent indole–PMMA, along with detectable amounts of the TAD–HDEO adduct (see Figure VI.10). Similarly, both PIBA- and PS-derived indole-functionalized polymers were clicked with BuTAD, followed by a thermal *transclick* reaction of the BuTAD group to HDEO.

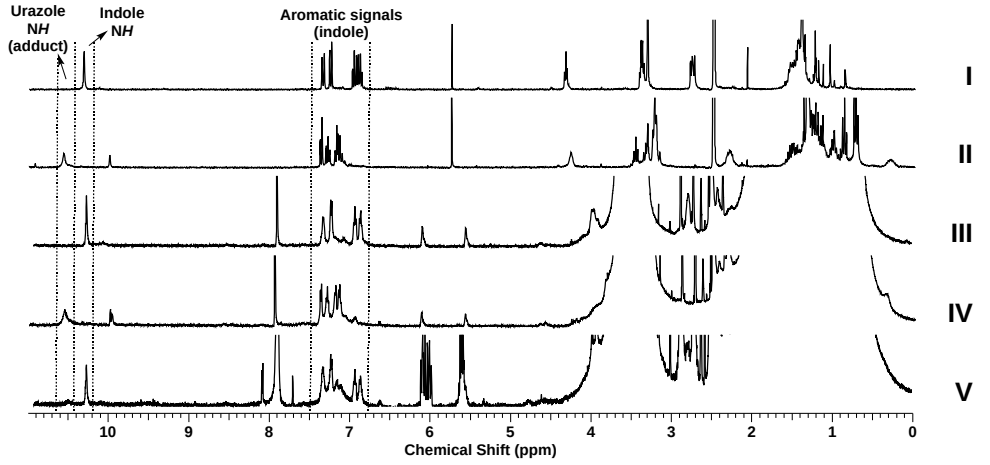


Figure VI.10: ^{1}H-NMR spectra of 2-*tert*-butyl-3-pentan-1-ol-1*H*-indole (indole-OH, I), indole-OH-BuTAD Adduct (II) and PMMA-Indole (III). After reaction of BuTAD with PMMA-Indole, spectrum IV is obtained. After heating to 120 °C for 30 minutes, the original spectrum (V) is obtained.

The successful results obtained in *clicking* and *transclicking* of a small TAD was then transposed to the synthesis of block copolymers. As in chapter IV, the TAD-functionalized polybutylacrylate was prepared with the aid of a urazole-derived alkyl bromide initiator,

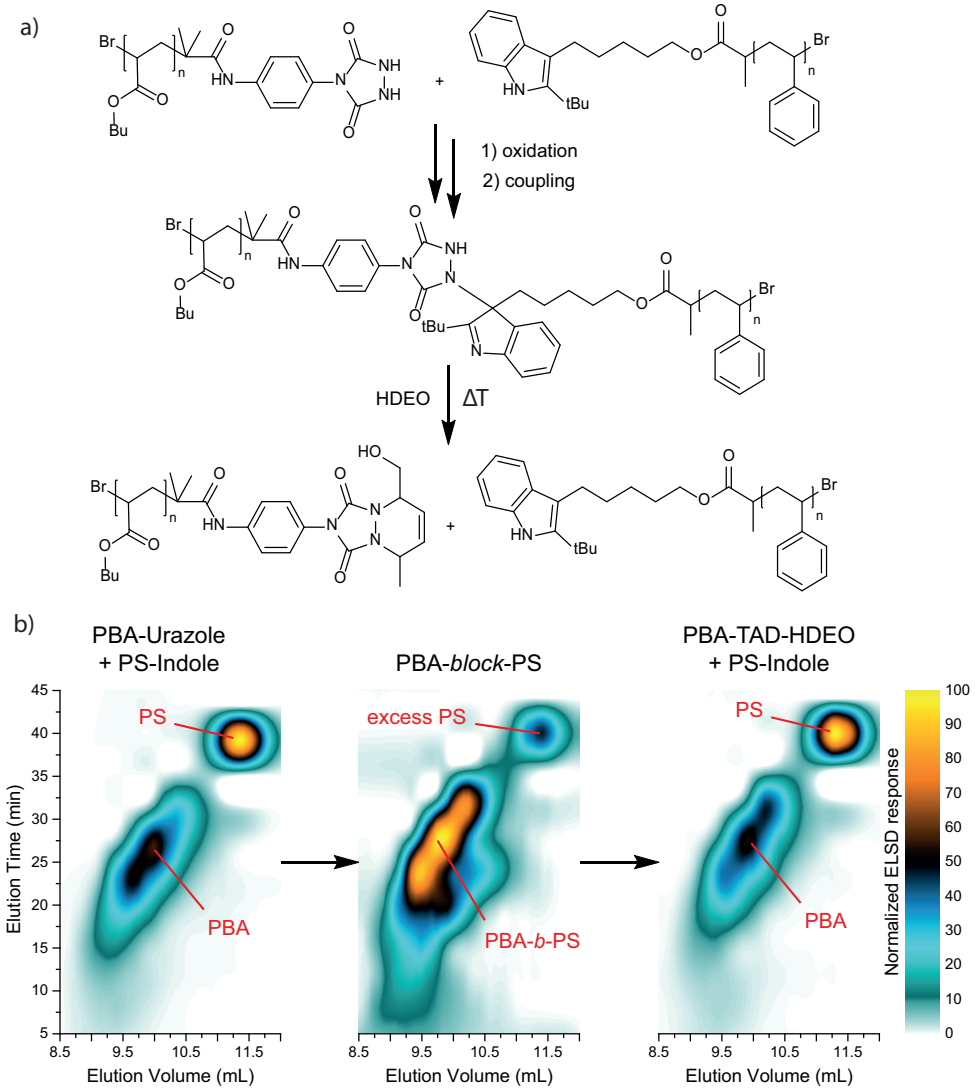


Figure VI.11: a) Polybutylacrylate-urazole (PBA-Urazole) is oxidized and reacted with polystyrene-indole to form the block copolymer. After heating the block copolymer with an excess of HDEO (120 °C for two hours in DMF), the original polymers are obtained and b) LCx-SEC analysis. Left: an equimolar amount of two separate polymers (PBA–urazole and PS–indole) before coupling. Centre: the block copolymer after coupling and right: after heating in the presence of excess HDEO.

via Cu-mediated radical polymerization (PBA–urazole, Mn = 20 000 g/mol, $Đ$ = 1.38). Again, this PBA-urazole was oxidized prior to use (in DCM with DABCO-Br for two hours), to give the TAD-endcapped polymer (PBA-TAD). This polymer was mixed with a polystyrene (PS) with an indole end group (Mn = 1400 g/mol, $Đ$ = 1.17) in THF. This gave the anticipated block copolymer formation within 30 minutes, as judged by the observed colour change and further confirmed by ^{1}H NMR analysis. The remarkably slower reaction rate in this case can be readily rationalized by the relatively low molar concentrations for both macromolecular reaction partners. LCx-SEC analysis was somewhat

hampered by a much higher detector response for the PS-based polymers (Figure VI.11, left). The elugram after the reaction (Figure VI.11, centre) also showed the presence of a residual amount of PS–indole. Furthermore, the larger difference in molecular weight for the two blocks (1400 g/mol compared to 20 000 g/mol for the PBA block) does not result in a significant change in elution time or elution volumes relative to the PBA homopolymer. However, when the block copolymer was treated with an excess of HDEO and heated to 120 °C in N,N-dimethylformamide (DMF) for two hours, the relative intensities in the resulting elugram (Figure VI.11, right) revealed the recovery of the original homopolymer distributions (Figure VI.11, left), which indicates that a selective *transclick* of one of the two initially *clicked* polymers had occurred.

VI.5 Synthesis of dynamic polymer networks based on reversible TAD-indole *click* linkages

Based on the previous encouraging results of the TAD-indole *click* and *transclick* reactions, a first attempt was made to bring this reversibility to polymer networks. For ease of comparison, again a simple polyurethane network was prepared (cf. section IV.5), this time containing an indole comonomer (**90** - see Figure VI.4) instead of a diene moiety. Again, two methods were used to obtain the materials. In the first one, crosslinking of a linear polyurethane (comprised out of hexamethylene diisocyanate (HDI), polypropylene oxide (PPO) and the indole diol (**90**)) was obtained by the addition of a bifunctional TAD molecule (**9**). The second approach made the crosslinks by reacting the indole diol and bisTAD, prior to polymerization. Both approaches gave similar end materials.

As a preliminary exploration of the possibilities of TAD-based *click* and dynamic *transclick* chemistries in functional materials, a number of qualitative tests were conducted with the materials obtained above. In a first test, a macroscopic scratch was made in the rubbery TAD-crosslinked PU networks. After heating the sample at 120 °C for one hour, the indole-derived sample was visibly healed, and it broke elsewhere after the application of stress. This result was further confirmed using a profilometer on the same material in which a scratch with a depth of 3 μm had been applied (Figure VI.12a). In another test, the material was broken into smaller pieces and put into a mold under pressure for 30 minutes

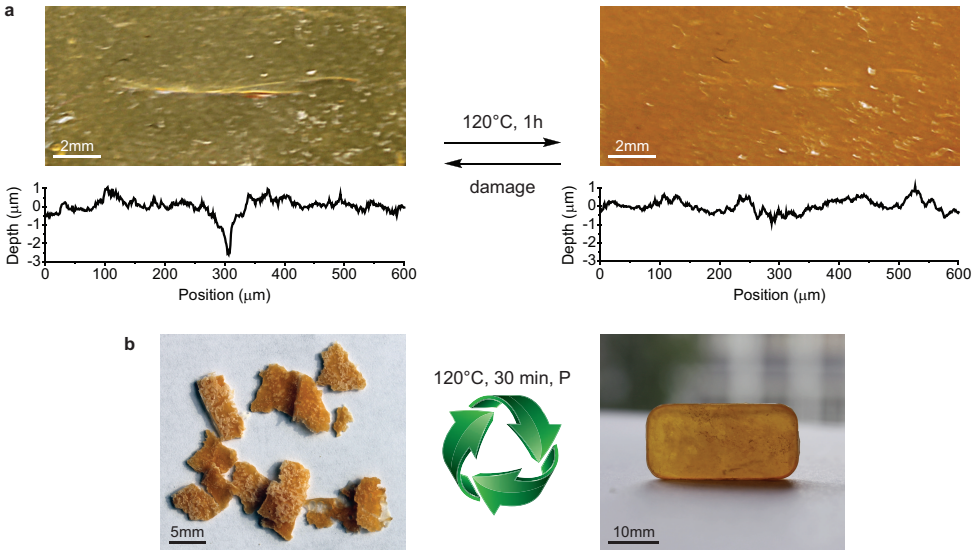


Figure VI.12: a) Image of a manually damaged PU sample (left) and the healed sample after heating for one hour at 120 °C (right) and measured profiles for a 3 μm deep scratch on another sample of an identical material and b) image of crosslinked PU sample that was broken into smaller pieces (left) and the same sample after being put into a mold under pressure (P) for 30 minutes at 120 °C (right).

at 120 °C. After cooling a pristine sample was retrieved from the mold (Figure VI.12b). This last test was repeated seven times, and each sample showed a similar behaviour of the storage modulus (Figure VI.13).Finally, this thermally assisted polymer-network reshaping, moulding and recycling was also demonstrated on a network derived from a PMMA copolymer with a high glass-transition temperature (T_g = 101 °C), which was prepared from the copolymerization of MMA with an indole-functionalized methacrylate comonomer (see). This stiff material could be moulded thermally and reprocessed in the same way as the PU rubbers. Moreover, it could also be powdered and moulded into a homogeneous material by extrusion above 110 °C (see Figure VI.14).

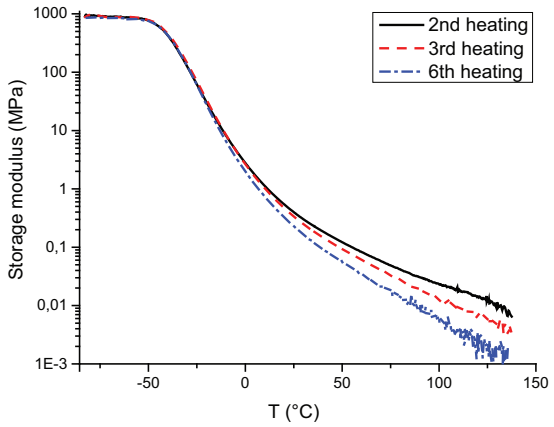


Figure VI.13: Dynamic mechanical thermal analysis thermograms (storage modulus versus temperature) for these PU networks after the second, third and sixth heating.

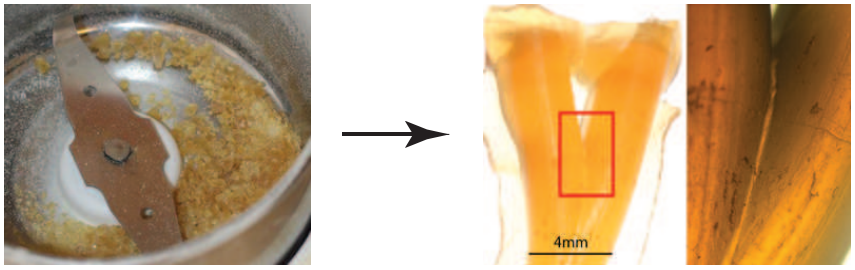


Figure VI.14: Initial extrusion experiment of TAD-indole crosslinked PMMA. The hard PMMA material was powdered and then fed into a twin screw miniature extruder set at 110 °C. Right hand images are of extrudate showing flow and fusion of the material coming from two channels in the moulding die.

VI.6 Conclusions and perspectives

We demonstrated in chapter IV that TAD chemistry can be labelled as *click* chemistry and that is has the ability to have its kinetic behaviour tuned or modulated by carefully selecting the complementary partner. Besides this interesting tunability, the possibility to control the reversibility of formed chemical bonds is of great interest as well (especially in polymer science). This chapter investigated the reactions between triazolinediones and 1*H*-indoles for their possibilities as dynamic covalent bond forming processes.

In a first step, the *click* characteristics for the coupling between TAD and 1*H*-indoles were investigated both by model studies and theoretical calculations. This was followed by a series of reversibility tests to determine the typical temperatures required for the retro reaction. Here it was noticed that a chemical trapping agent for free TAD molecules was necessary, due to the fast re-bonding of the starting compounds at lower temperatures preventing significant or measurable build-up of free TAD moieties. In this context, we have chosen for the ultrafast irreversible Diels-Alder reactions that were described in the chapter IV. Surprisingly, this kinetic trapping experiment appeared to be a very clean TAD-transfer process from indole to diene groups. Thus, the term *transclick* reaction was introduced. After the model studies, this newly introduced concept was tested on macromolecular level. First, a block copolymer was synthesized based on TAD-indole chemistry. This block copolymer showed its reversible nature by releasing the two original polymers upon heating in the presence of the required trapping molecule. In a last stage, a variety of polymer networks was synthesized showing clear dynamic behaviour in simple applications such as healing, recycling and reshaping.

This chapter showed that TAD-indole *click* chemistry has the ability to show reversibility at elevated temperature and can undergo very efficient *click-like* group transfer processes. To protect this 'new' type of reactivity, a patent application was filed that was granted in the beginning of 2015.[10] Ongoing industrial projects give the PCR group the opportunity to further examine the valorisation potential of this reversible TAD-based chemistry.

VI.7 Experimental section

VI.7.1 Materials

Aluminiumoxide (activated, basic), 2,2-bis(hydroxymethyl)propionic acid (98%), α-bromo-isobutyryl bromide (98%), butyl acrylate (>99%), Cu(0) was used as pellets (>99.999%), copper(I)bromide (99%), copper(I)iodide (98%), celite®, dibutyltin dilaurate (95%), 3,4-dihydro-2*H*-pyran (97%), tris[2-(dimethylamino)ethyl]amine (Me_6TREN, 97%), 2,3-di-methyl-2-butene (99%), *trans,trans*-2,4-hexadien-1-ol (>97%), lithium aluminium hydride (LAH, powder, 95%), methanesulfonyl chloride (99%), 3-methylbutyraldehyde (97%), 4,4'-methylenebis(phenyl isocyanate) (98%), 4-nitrophenyl isocyanate (97%), palladium on carbon (5%), N,N,N',N",N"-pentamethyldiethylenetriamine (PMDETA, 99%), potassium hydroxide (reagent grade, 90%, flakes), sodium iodide (>99.5%), *N,N,N',N'*-tetramethylethylenediamine (TMEDA, 99%) and trifluoro-acetic acid (99%) were purchased from Sigma-Aldrich. Butyl isocyanate (>98%) and dibutyltin dilaurate from TCI, hydrochloric acid (36%) from Chem-Lab. Ammonium chloride (99%), potassium carbonate (99%) and sodium bicarbonate (99.5%) from Roth. Hexamethylene diisocyanate(>98%) from Fluka, magnesium sulphate (anhydrous) from Boom. Ethyl carbazate (97%), polypropylene oxide ($M_n \approx 2000$ g/mol) and sodium hydroxide (97%) from Acros Organics. All solvents (Sigma-Aldrich) and products were used without any pre-treatment or purification.

Styrene (Sigma-Aldrich, ≥99%) was passed over a short column of Al_2O_3 prior to use. *N,N,N',N",N"*-pentamethyldiethylenetriamine (PMDETA, Sigma-Aldrich, 99%) was distilled (85 −86 °C, 16 mbar) and stored at 4 °C. Cu(I)Br (Sigma-Aldrich, 98%) was purified by stirring with acetic acid, then by filtering and washing with ethanol and diethylether, and finally by drying in a vacuum oven at 70 °C.

VI.7.2 Characterization

Nuclear Magnetic Resonance (NMR) ^{1}H-spectra were recorded with a Bruker Avance 300 (300 MHz) FT-NMR spectrometer in $CDCl_3$ (Eurisotop) or DMSO-d_6 solution at room temperature. Chemical shifts are presented in parts per million (δ) relative to $CHCl_3$ (7.26 ppm for ^{1}H-NMR) and DMSO (2.50 ppm for ^{1}H-NMR) as an internal standard. The

resonance multiplicities are described as [br. (broad)] s (singlet), d (doublet), t (triplet), q (quadruplet), quin (quintuplet), sext (sextuplet) or m (multiplet).

Thermogravimetric analysis (TGA) was performed using a Mettler-Toledo TGA / SDTA 851e equipment. Samples (5 to 10 mg) were heated in a nitrogen atmosphere with a heating rate of 10 K min^{-1} going from 25 °C to 600 °C. For the analysis of the thermograms, the STARe software of Mettler-Toledo was used.

Differential Scanning Calorimetry (DSC) thermograms were recorded using a TA Instruments Q2000 DSC with autosampler option and Refrigerated Cooling System (RCS). Nitrogen gas was used as purge gas. The samples were studied in TAi Tzero Hermetic aluminium sample pans and at a scan rate of 10 K min^{-1}.

Dynamic mechanical analysis (DMA) was performed from −80 to 70 °C, in a TA Instrument (DMA 2980) at 10 K min^{-1} with a frequency of 1 Hz

Liquid Chromatography - Mass Spectrometry (LC-MS) analyses were performed on an Agilent Technologies 1100 series LC/MSD system with a diode array detector (DAD) and a single quad MS. Analytical reversed phase HPLC-analyses were performed with a Phenomex Luna C18 (2) column (5 μm, 250 mm × 4.6 mm) and a solvent gradient (0-100 % acetonitrile in H_2O in 15 min). The eluted compounds were analyzed via UV detection (214 nm).

LCx–SEC analysis. For two-dimensional LC, sample fractions from the first dimension were transferred to the second-dimension column via an electronically controlled eight-port valve system (VICI Valco instruments), equipped with two 200 µL sample loops. The second dimension consisted of an Agilient Infinity 1260 isocratic pump and a PSS SDV LIN M 200 µm column. Detection in the second dimension was accomplished by using an evaporative light-scattering detector (ELSD). Nitrogen was used as the carrier gas in the ELSD at a flow rate of 2.5 L/min. Spray chamber, drift tube and optical cell temperatures were set at 30 °C, 80 °C and 70 °C, respectively. The flow rates used in the first and second dimensions were 0.05 mL/min and 5 mL/min, respectively. Sample concentrations were between 0.25 and 2.0 mg/mL. THF was used as the solvent for the second dimension analysis. Data were recorded using PSS WinGPC Unichrom software.

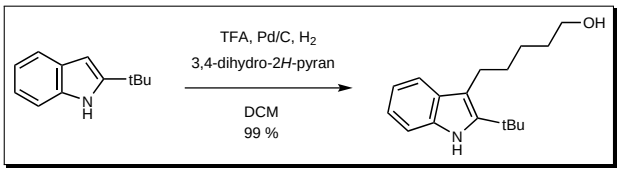
Computational methodology All measurements were performed by Dr. Hannelore Goossens of the *Centre of Molecular Modeling* of *Ghent University*. To start with, the B3LYP/6-31+G(d,p) level of theory was used for geometry optimizations. A thorough conformational analysis was performed on all reactants, transition states, intermediates and products to identify the most plausible conformers. The stationary points were characterized as minima (ground states) or first order saddle points (transition states) by normal modes analysis. Intrinsic reaction coordinate (IRC) calculations were used to verify the corresponding reactant and product complexes. The B3LYP functional has been proven to produce good geometries but is less accurate for energy calculations. Therefore energies were refined with the M06-2X functional, which is able to account for dispersion effects, and a 6-31++G(d,p) basis set. Since the studied reactions take place in dichloromethane, which cannot form hydrogen bonds with the reactive substrate, the solvent environment was taken into account by means of a continuum model. From preliminary calculations, where a polarizable continuum model (PCM) was only taken into account for energy refinement, it was found to be necessary to take PCM into account for geometry optimizations. Thermal free-energy and enthalpy corrections were taken from B3LYP/6-31+G(d,p) optimizations at 1 atm and 298.15 K. Next, the M06-2X/6-31+G(d,p) level of theory, in combination with PCM, was used for geometry optimizations of the most plausible reaction paths, since dispersion effects were assumed to play an important role in the reactions. All computations were carried out with the Gaussian 09 program package.

VI.7.3 Synthesis

The synthesis of the following compounds can be found in previous chapters:

- 4-Butyl-1,2,4-triazoline-3,5-dione (BuTAD, **31**) - see III.8.3.1
- 1,4-Diazabicyclo[2.2.2]octane bromide complex (DABCO-Br) - see III.8.3.2
- 2-*tert*-Butyl-3-isopentyl-1*H*-indole (**34**) - see III.8.3.3
- Urazole initiator for Cu(0) mediated polymerization (**56**) - see III.8.3.9
- 4,4'-(4,4'-Diphenylmethylene)-bis-(1,2,4-triazoline-3,5-dione) (**9**) - see IV.7.3.2
- Polybutylacrylate-Urazole (PBA-Urazole) - see IV.7.3.10

VI.7.3.1 Indole-OH (89)



A mixture of trifluoro-acetic acid (7.91 g, 69.2 mmol, 1.5 equivalent), palladium (5% on activated carbon, 0.7 g) and dichloromethane (95 mL) was put under hydrogen atmosphere in a 500 mL two neck flask and cooled in an ice bath. To this mixture, 2-*tert*-butyl-1*H*-indole (8 g, 46.2 mmol, 1 equivalent) and 3,4-dihydro-2*H*-pyran (4.3 g, 51.1 mmol, 1.1 equivalent) in dichloromethane (140 mL) were added dropwise. This solution was stirred in a water bath for 48 hours, regularly flushing with hydrogen gas. With the help of TLC (hexane:ethyl acetate 9:1) the reaction was followed until completion. The mixture was filtered over celite and washed with saturated aqueous sodium bicarbonate solution (100 mL). The organic phases are dried over magnesium sulphate and concentrated *in vacuo* to obtain a yellow brown oil. To this oil, methanol was added until a dilution of 10 m%, followed by the addition of potassium carbonate until saturation of the solution. After stirring for 15 minutes, water was added to double the volume. The obtained mixture was concentrated until half volume under reduced pressure and extracted with dichloromethane (2 times 100 mL). The obtained organic phases were collected, dried on magnesium sulphate and concentrated *in vacuo* to yield 11.9 g yellow brown oil (99%).

Bruto formula: $C_{17}H_{25}NO$.

MW.: 259.39 g/mol.

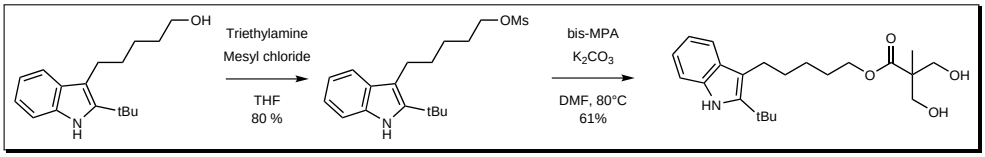
MS (m/z): experimental: 260.2

HRMS (m/z for [MH]+): calculated: 260,2009, experimental: 260.2017

^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) = 1.38 (s, 9 H, $C(CH_3)_3$), 1.45 (m, 2 H, $CH_2-CH_2-CH_2$), 1.59 (m, 4 H, $CH_2-CH_2-CH_2$), 2.79 (t, 2 H, C=C−CH_2), 3.59 (t, 2 H, CH_2−OH), 7.01 (m, 2 H, Ar*H*), 7.20 (d, 1 H, Ar*H*), 7.42 (d, 1 H, Ar*H*), 7.76 (br.s, 1 H, N*H*).

Reference: Cao L.L., Wang D.S., Jiang G.F., Zhou Y.G., Tetrahedron Letters, 2011, 52, 2837-2839.

VI.7.3.2 Indole-diol (90)



In a two neck 250 mL flask, 2-*tert*-butyl-3-pentan-1-ol-1*H*-indole (11.9 g, 45.9 mmol, 1 equivalent) is dissolved in anhydrous THF (120 mL). After addition of anhydrous triethylamine (9.29 g, 91.8 mmol, 2 equivalents) this mixture was cooled in an ice bath. Then methane sulfonylchloride (5.33 mL, 68.9 mmol, 1.5 equivalents) was added dropwise and the reaction was stirred for three hours under inert atmosphere. With the aid of TLC (hexane:ethylacetate 1:1) the complete conversion of the starting product was checked. The mixture was washed with water (60 mL), the obtained water phase was extracted with THF (60 mL). The combined organic phases were eventually washed with saturated aqueous sodium carbonate solution (2 x 60 mL) and brine (60 mL). After drying over magnesium sulphate, the mesylate (12.4 g – 80%) was acquired after concentrating the organic solvent *in vacuo*.

Following this, a mixture of 2,2-bis-(hydroxymethyl)-propionic acid (1.99 g, 14.8 mmol, 1 equivalent), potassium carbonate (2.25 g, 16.3 mmol, 1.1 equivalent) and anhydrous dimethylformamide (DMF, 15 mL) was placed in a two neck flask (50 mL) under an inert atmosphere and cooled in an ice bath. To this mixture, a solution of 2-*tert*-butyl-3-pentan-1-mesylate-1*H*-indole (5.00 g, 14.8 mmol, 1 equivalent) in anhydrous DMF (15 mL) was added. This mixture was stirred vigorously overnight at 80 °C. With the aid of TLC (hexane:ethyl acetate 1:1) the complete convergence of the starting product was checked. The salts were filtered off and washed with DMF, after which the filtrate was concentrated under reduced pressure. To the acquired orange brown oil 5% triethylamine in ethyl acetate was added to a dilution of 10 m%. This mixture was then placed in an ultrasonic bath for 10 minutes. The salts were filtered off and washed with 5% triethylamine in ethyl acetate, after which the filtrate was concentrated *in vacuo* to obtain 5-(2-*tert*-butyl-1*H*-indole-3-yl)-pentyl-2,2-bis-(hydroxymethyl)-propanoate. This product was purified via chromatography (gradient: hexane:ethyl acetate 4:1 ; hexane:ethyl acetate 1:1) to yield the pure product as an orange brown oil (3.38 g, 61%).

Bruto formula: $C_{22}H_{33}NO_4$.

MW.: 375.51 g/mol.

MS (m/z): experimental: 376.2

HRMS (m/z for [MH]+): calculated: 376,2482, experimental: 376.2487

^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) = 1.06 (s, 3 H, CO−C−CH_3), 1.47 (s, 9 H, C(CH_3)$_3$), 1.53 (m, 2 H, CH_2−CH_2−CH_2), 1.73 (m, 4 H, CH_2−CH_2−CH_2), 2.81 (t, 2 H, CH_2−OH), 2.88 (t, 2 H, C=C−CH_2), 3.71 (dd, 2 H, CH_2−OH), 3.90 (dd, 2 H, CH_2−OH), 4.20 (t, 2 H, CH_2−O−CO), 7.10 (m, 2 H, ArH), 7.29 (d, 1 H, ArH), 7.50 (d, 1 H, ArH), 7.85 (br.s, 1 H, NH).

VI.7.3.3 Determining the reversibility temperature of TAD-indole adducts

The reversiblity temperature of all involved TAD-indole adducts were determined via the same procedure described in VI.2.2. model compounds were obtained via the same method. Although the some NMR measurements suggest that not all indole components can be recovered from the adduct (curve does not always reaches 100%), this is not in agreement with the LC-MS analysis. It was shown that no adduct could be detected after heating to 160 °C (see Figure VI.7). In the case of indole-OH and indole-diol, the maximal amount of liberated indole is 97 and 96%, which is still within the error margin of the NMR integrations. In case of the non-functional *i*-pentylindole, a maximum of 88% is reached. This can however be explained by the NMR spectra. After heating to 160 °C there remains, in the case of indole diol, still a significant quantity of unreacted *trans*,*trans*-2,4-hexadien-1-ol (HDEO) in the solution (signal ***(H-5)*** and ***(H-6)*** in Figure VI.6), which is in agreement with the weighed amount of HDEO (1.1 equivalent). These same signals were not visible in the spectrum of *i*-pentylindole after heating to 160 °C. This suggest that – due to a mistake in measuring – not enough HDEO was present, which limits the maximal amount of liberated indole. When all the HDEO is consumed, the liberated 4-butyl-1,2,4-triazoline-3,5-dione (BuTAD) will reconnect with the indole upon cooling. To clarify this point, the fraction of released indole (or percentage of transclick) was normalized in Figure VI.8.

- ***ene* adduct (91) of indole-diol (90)-indole and BuTAD**

  ^{1}H-NMR (300 MHz**, DMSO−d_6):** δ (ppm) = 0.73 (t, 3 H, CH_3−CH_2−CH_2−CH_2), 0.99 (s, 3 H, CH_3−C), 1.03 (m, 2 H, CH_3−CH_2−CH_2−CH_2), 1.17 (m, 6 H, CH_3−CH_2−CH_2−CH_2 + C(O)−O−CH_2−CH_2−CH_2−CH_2), 1.33 (m, 11 H, C(CH_3)$_3$ + CH_2−CH_2−O−C(O)), 2.32 (t, 2 H, CH_2−C−N−NH), 3.22 (m, 2 H, CH_3−CH_2−CH_2−CH_2), 3.84 (dd, 2 H, HO−CH_2), 3.93 (dd, 2 H, HO−CH_2), 4.02 (t, 2 H, CH_2−O−C(O)), 4.63 (br s , 2 H, OH), 6.75-7,5 (band, 4 H, ArH), 10.60 (br s , 1 H, NH).

- **DA adduct (33) of *trans*,*trans*,-2,4-hexadien-1-ol and BuTAD**

  ^{1}H-NMR (300 MHz**, DMSO−d_6):** δ (ppm) = 0.88 (t, 3 H, CH_3−CH_2−CH_2−CH_2), 1.27 (sext, 2 H, CH_3−CH_2−CH_2−CH_2), 1.33 (d, 3 H, CH_3−CH), 1.52 (quin, 2 H, CH_3−CH_2−CH_2−CH_2), 3.39 (t, 2 H, CH_3−CH_2−CH_2−CH_2), 3.65 (br d, 1 H, HCH−OH), 3.77 (br d, 1 H, HCH−OH), 4.30 (m, 2 H, CH−N−N−CH), 5.00 (br s, 1 H, HO−CH_2), 5.91 (s, 2 H, CH=CH).

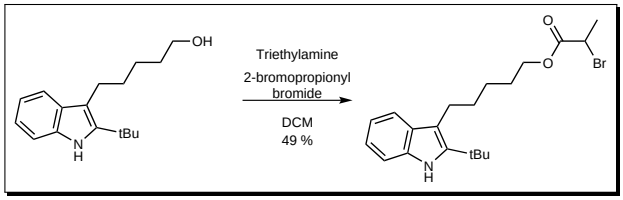
- ***ene* adduct of 2-*tert*-butyl-3-isopentyl-1*H*-indole (34) and BuTAD**

  ^{1}H-NMR (300 MHz**, DMSO−d_6):** δ (ppm) = 0.73 (m, 6 H, CH(CH_3)$_2$ + CH_3−CH_2−CH_2−CH_2), 0.89 (m, 2 H, CH_3−CH_2−CH_2−CH_2), 1.03 (m, 2 H, CH_3−CH_2−CH_2−CH_2), 1.32 (s, 9 H, C(CH_3)$_3$), 1.71 (dd, 3 H, CH_2−CH(CH_3)$_2$), 2.29 (m, 2 H, NH−N−C−CH_2), 3.24 (t, 2 H, CH_2−CH_2−CH_2−CH_3), 6.75-7,5 (band, 4 H, ArH), 10.62 (s, 1 H, NH).

- ***ene* adduct of indole-OH (89) and BuTAD**

  ^{1}H-NMR (300 MHz**, DMSO−d_6):** δ (ppm) = 0.73 (t, 3 H, CH_3−CH_2−CH_2−CH_2), 1.01 (m, 2 H, CH_3−CH_2−CH_2−CH_2), 1.21 (m, 4 H, CH_3−CH_2−CH_2−CH_2 + CH_2−CH_2−CH_2−OH), 1.33 (s, 9 H, C(CH_3)$_3$), 1.51 (m, 4 H, CH_2−CH_2−OH + NH−N−C−CH_2−CH_2), 2.26 (t, 2 H, CH_2−C−N−NH), 3.24 (m, 4 H, CH_3−CH_2−CH_2−CH_2 + HO−CH_2), 6.75-7,5 (band, 4 H, ArH), 10.61 (br s , 1 H, NH).

VI.7.3.4 5-(2-tert-butyl-1H-indole-3-yl)pentyl-2-bromopropanoate (93)



In a two neck 25 mL flask a solution of 2-tert-butyl-3-pentan-1-ol-1H-indole (2 g, 7.71 mmol, 1 equivalent) and anhydrous triethylamine (1.17 g, 11.57 mmol, 1.5 equivalent) in anhydrous dichloromethane (10 mL) was placed under inert atmosphere. After cooling down in an ice bath, 2-bromopropionyl bromide (1.01 ml, 9.64 mmol, 1.25 equivalent) was added dropwise and stirred overnight at room temperature. With the aid of TLC (hexane: ethyl acetate 1:1) the disappearance of the starting alcohol was followed. Salts were filtered off and the filtrate was washed with water (2 x 10 mL) and saturated aqueous sodium carbonate solution (3 x 10 mL). After drying on magnesium sulphate the initiator (1.50 g, 49%) was obtained by concentrating the organic solvent phase in vacuo.

Bruto formula: $C_{20}H_{28}BrNO_2$.

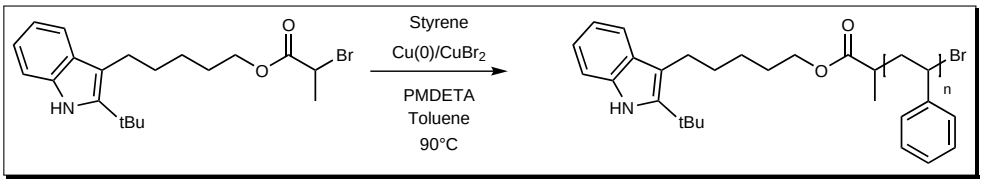
MW.: 394.35 g/mol.

MS (m/z): experimental: 394.1

HRMS (m/z for [MH]+): calculated: 394,1376, experimental: 394.1375

^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.39 (s, 9 H, C(CH_3)$_3$)), 1.47 (m, 2 H, CH_2−CH_2−CH_2), 1.64 (m, 4 H, CH_2−CH_2−CH_2), 1.75 (d, 3 H, CH_3−CHBr), 2.79 (m, 2 H, C=C−CH_2), 4.12 (m, 2 H, CH_2−O), 4.30 (q, 1 H, Br−CH), 7.01 (m, 2 H, ArH), 7.21 (d, 1 H, ArH), 7.42 (d, 1 H, ArH), 7.75 (br.s, 1 H, NH).

VI.7.3.5 Polystyrene-Indole (PS-In)

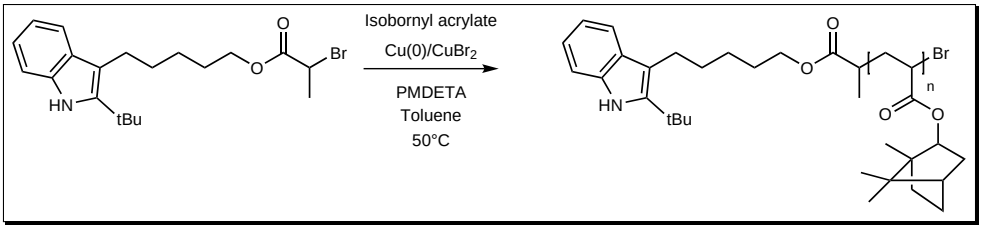


Styrene (15 mL, 130.5 mmol, 100 equivalents), toluene (10 mL), Cu(0) (30 pellets), Cu(II)Br_2 (29.14 mg, 130.49 μmol, 0.1 equivalent) and N,N,N',N",N"-pentamethyldiethylenetriamine (PMDETA, 95.36 μL, 0.46 mmol, 0.35 equivalent) were weighed into

a flask and degassed for one hour with a continuous nitrogen sparge. A solution of 5-(2-tert-butyl-1*H*-indole-3-yl)pentyl-2-bromepropanoate (**93**, 514.57 mg, 1.30 mmol, 1 equivalent) in toluene (5 mL) was degassed separately in an ampule by nitrogen sparge for one hour. After addition of the initiator to the reaction mixture, the flask was placed in an oil bath at 90 °C and the reaction started. After four hours, the reaction was stopped (18% conversion) by cooling in liquid nitrogen under air atmosphere and precipitation in cold methanol (300 mL). The precipitate was filtered off and dissolved in tetrahydrofuran (100 mL). The copper catalyst was removed by passing the reaction mixture over a column of Al_2O_3. After evaporating the excess solvent until a volume of 20 mL, the polymer was precipitated in cold methanol (200 mL). The polymer was filtered, washed with methanol and again dissolved in THF (20 mL). Finally the polymer was precipitated in cold methanol (200 mL) and dried overnight in a vacuum oven at 40 °C.

$\boldsymbol{M_n}$ **(SEC)**: 1400 g mol^{-1}, $\boldsymbol{M_w}$ **(SEC)**: 1600 g mol^{-1}, $\boldsymbol{Đ}$ **(SEC)**: 1.17

VI.7.3.6 Polyisobornylacrylate-Indole (PiBA-In)



Isobornyl acrylate (15 mL, 71 mmol, 100 equivalents), N,N,N',N",N"-pentamethyldiethylenetriamine (PMDETA, 296.5 μL, 1.42 mmol, 2 equivalents) and Cu(I)Br (203.7 mg, 1.42 mmol, 2 equivalents) were weighed into a flask and degassed for one hour with a continuous nitrogen sparge. Afterwards, a degassed solution of 5-(2-tert-butyl-1H-indole-3-yl)pentyl-2-bromepropanoate (**93**, 280 mg, 0.71 mmol, 1 equivalent) in acetone (5 mL) was added and the reaction mixture was placed in an oil bath at 50 °C. After three hours, the reaction was stopped (12 % conversion) by cooling in liquid nitrogen under air atmosphere and precipitation in cold methanol (200 mL). The precipitate was filtered off and dissolved in THF (100 mL). The copper catalyst was removed by passing the reaction mixture over a column of Al_2O_3. After evaporating the excess solvent until a volume of 20 mL, the polymer was precipitated in cold methanol (200 mL). The polymer was filtered, washed with methanol and again dissolved in tetrahydrofuran (20 mL). Finally,

the polymer was precipitated in cold methanol (200 mL) and dried overnight in a vacuum oven at 40 °C.

M_n (SEC): 1900 g mol^{-1}, **M_w (SEC)**: 2300 g mol^{-1}, **$Đ$ (SEC)**: 1.22

VI.7.3.7 General procedure for oxidizing polymers with urazole end group

1 mmol of polymer with an urazole end group is dissolved in 5 mL of dichloromethane. To this 0.3 mmol of DABCO-Br is added at room temperature. The solution was allowed to stir for three hours at room temperature. Then the solution was filtrated and concentrated *in vacuo* to obtain the polymer with a TAD end group.

VI.7.3.8 General procedure for polymer-polymer conjugation

The polymer (50 mg, 1 equivalent) with an Cp/Indole end group was dissolved in tetrahydrofuran (THF, 0.5 mL). To this, a solution of TAD polymer in THF (0.5 mL, one equivalent) was added at room temperature. The solution was allowed to stir until the red colour disappeared. The obtained block copolymer was precipitated in the appropriate solvent, filtrated, washed thoroughly and dried overnight in a vacuum oven at 40 °C.

VI.7.3.9 Making a reversible network from linear polyurethane

1.00 g of the indole-containing polyurethane was dissolved in dimethylformamide (5mL) and to this was added a solution of 4,4'-(4,4'-diphenylmethylene)-bis-(1,2,4-triazoline-3,5-dione) (**9**, 58.2 mg, 0.161 mmol, 0.25 equivalent) in dimethylformamide (1 mL). After the reaction went to completion (in about two minutes, as judged by the disappearance of the red colour), the network was dried overnight in a vacuum oven at 40 °C.

T_g (DSC): −45 °C, **T_{deg} (TGA)**: 300 °C.

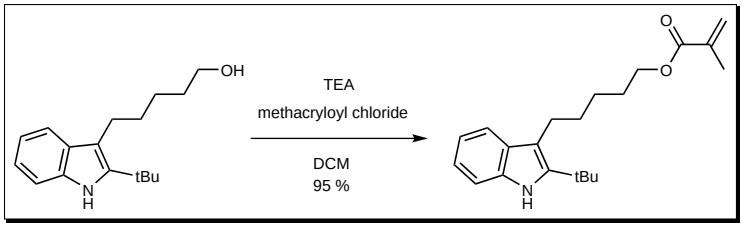
VI.7.3.10 Direct synthesis of the reversible polyurethane network

To a solution of indole diol (0.805 g, 2.14 mmol, 0.68 equivalent) in dimethylformamide (3 mL) was added 4,4'-(4,4'-diphenylmethylene)-bis-(1,2,4-triazoline-3,5-dione) (**9**, 0.194 g, 0.536 mmol, 0.17 equivalent). After the reaction had gone to completion (less than one minute, disappearance of the red colour), the solution was mixed with polypropylene oxide (2.00 g , 1.00 mmol, 0.32 equivalent) and hexamethylene diisocyanate (0.529 g, 3.15

mmol, 1 equivalent) in a 50 mL flask. Then dibutyltin dilaurate (29 μL, 0.049 mmol, 0.01 equivalent) was added and the mixture was well stirred. This reactive mixture was injected between two glass plates, separated by a silicone spacer and placed in an oven at 70 °C for 6 hours. The obtained network was dried overnight in a vacuum oven at 40 °C.

T_g (DSC): −49 °C, **T_{deg} (TGA)**: 300 °C.

VI.7.3.11 Synthesis of indole methacrylate



In a 250 mL two neck flask 2-*tert*-butyl-3-pentan-1-ol-1*H*-indole (11,9 g, 45.9 mmol, 1 equivalent) was dissolved in dry dichloromethane (100 mL). After the addition of dry triethylamine (5,81 g, 57.4 mmol, 1.25 equivalent), the mixture was cooled in an ice bath. In a next step, methacryloyl chloride (6.00 g, 57.4 mmol, 1.25 equivalent) in dry dichloromethane (50 mL) was added dropwise and the mixture was stirred under inert atmosphere overnight. With the aid of TLC (hexane:ethyl acetate 1:1) the complete conversion of the starting product was checked. The reaction mixture was filtrated and to the filtrate was added sequentially saturated aqueous sodium carbonate solution (50 mL), water (150 mL) and dichloromethane (50 mL). The organic phase was washed with saturated aqueous sodium carbonate solution (100 mL), brine (10 mL) and water (100 mL). The water phase was extracted with dichloromethane (100 mL). The combined organic phases were dried over magnesium sulphate and concentrated *in vacuo* to obtain 5-(2-*tert*-butyl-1*H*-indole-3-yl)pentylmethacrylate (14.3 g, 95 %).

Bruto formula: $C_{21}H_{29}NO_2$.

MW.: 327.46 g/mol.

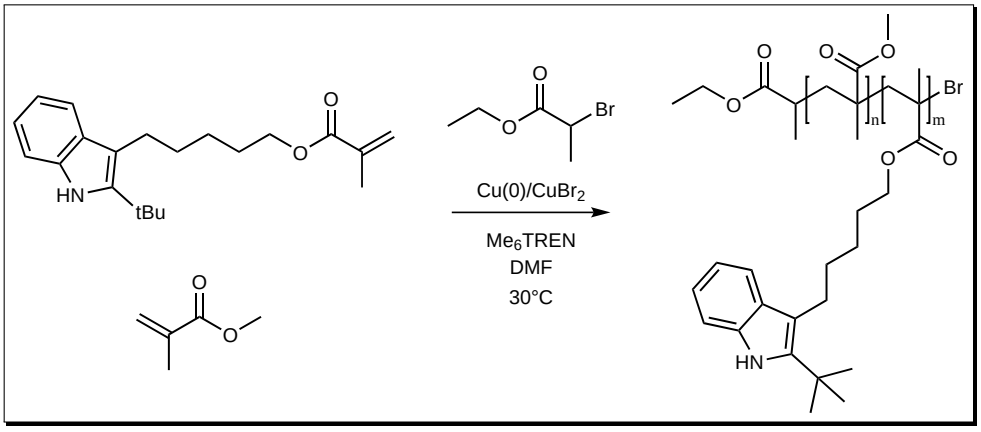
MS (m/z): experimental: 328.2

HRMS (m/z for [MH]+): calculated: 328.2271, experimental: 328.2276

^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) = 1.47 (s, 9 H, $C(CH_3)_3$), 1.55 (m, 2 H, $CH_2-CH_2-CH_2$), 1.72 (m, 4 H, $CH_2-CH_2-CH_2$), 1.96 (s, 3 H, $CO-C-CH_3$), 2.87 (t, 2 H, C=

C−CH_2), 4.18 (t, 2 H, CH_2−O), 5.56 (s, 1 H, CO−C=CH_2), 6.11 (s, 1 H, CO−C=CH_2), 7.09 (m, 2 H, ArH), 7.29 (d, 1 H, ArH), 7.50 (d, 1 H, ArH), 7.83 (br.s, 1 H, NH).

VI.7.3.12 Synthesis of poly(methylmethacrylate-co-indole-methacrylate)



Methyl methacrylate (5.0 mL, 46.9 mmol, 40 equivalents), indole methacrylate (3.84 g, 11.7 mmol, 10 equivalents), ethyl-2-bromo propionate (0.991 mL, 7.63 mmol, 6.5 eq) en Cu(0) (30 pellets) were mixed in a flask. Parallel a solution containing cupper(II)bromide (83.5 mg, 0.374 mmol, 0.3 equivalent) and Me_6TREN (300 μL, 1.12 mmol, 0.95 equivalent) in dimethylformamide (18.7 mL) was prepared. Both solutions were degassed during one hour by passing through nitrogen gas. Then the cupper(II)bromide solution was added to the monomer solution and stirred for 24 hours at 30 °C under inert atmosphere. The reaction was stopped by cooling the mixture with liquid nitrogen open to the air. The reaction mixture was then diluted with tetrahydrofuran to double volume, filtrated over basic aluminium oxide and concentrated *in vacuo* to a volume of about 20 mL. The polymer was precipitated in cold methanol (200 mL), filtrated, washed thoroughly with methanol and dried overnight in a vacuum oven at 40 °C (yield = 79%).

$\boldsymbol{M_n}$ **(SEC)**: 8900 g mol^{-1}, $\boldsymbol{M_w}$ **(SEC)**: 16 300 g mol^{-1}, $\boldsymbol{Đ}$ **(SEC)**: 1.84 , $\mathbf{T_g}$ **(DSC)**: 91 °C, $\mathbf{T_{deg}}$ **(TGA)**: 270 °C.

VI.7.3.13 Synthesis of reversible network based on Poly(MMA-co-indole-MA)

1.00 g of the poly(MMA-co-indole-MA), was dissolved in tetrahydrofuran (4 mL). To this was added 4,4'-(4,4'-diphenylmethylene)-bis-(1,2,4-triazoline-3,5-dione) (124 mg, 0.342 mmol, 0.25 equivalent) in tetrahydrofuran (1.5 mL). After the reaction has gone to completion (in about 3 minutes), the network was dried overnight in a vacuum oven.

T_g (DSC): 101 °C, **T_{deg} (TGA)**: 280 °C.

VI.8 Bibliography

[1] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angewandte Chemie International Edition* **2001**, *40*, 2004–2021.

[2] J. E. Moses, A. D. Moorhouse, *Chemical Society Reviews* **2007**, *36*, 1249–1262.

[3] W. Xi, T. F. Scott, C. J. Kloxin, C. N. Bowman, *Advanced Functional Materials* **2014**, *24*, 2572–2590.

[4] T. Pauloehrl, G. Delaittre, V. Winkler, A. Welle, M. Bruns, H. G. Börner, A. M. Greiner, M. Bastmeyer, C. Barner-Kowollik, *Angewandte Chemie International Edition* **2012**, *51*, 1071–1074.

[5] B. D. Mather, K. Viswanathan, K. M. Miller, T. E. Long, *Progress in Polymer Science* **2006**, *31*, 487–531.

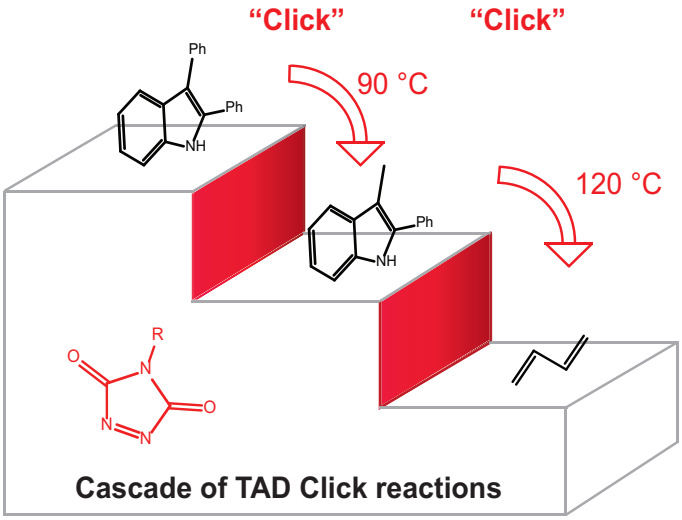
[6] P. S. Baran, C. A. Guerrero, E. J. Corey, *Organic Letters* **2003**, *5*, 1999–2001.

[7] H. Zweifel, R. Maier, M. Schiller, *Plastics Additives Handbook*, Hanser, 6th ed., **2009**.

[8] H. R. Kricheldorf, Z. Denchev, *Transreactions in Condensation Polymers*, **1999**.

[9] J. Zhou, N. K. Guimard, A. J. Inglis, M. Namazian, C. Y. Lin, M. L. Coote, E. Spyrou, S. Hilf, F. G. Schmidt, C. Barner-Kowollik, *Polymer Chemistry* **2012**, *3*, 628–639.

[10] S. Billiet, K. De Bruycker, J. Winne, F. E. Du Prez, *WO2015018928* **2015**, Urazole compounds.



Abstract:

The TAD-based *click* and *transclick* reactions described and introduced in previous chapters, provide unique further opportunities to develop highly tuned or 'programmed' *click* reactions. Tuning of the reversibility profile could indeed open the door to additional applications and improve the applicability of the dynamic *click* chemistry platform. For this reason, novel TAD-indole combinations are explored herein, that should have all the *click* characteristics as the original system but also possess different (lower) reversibility temperatures. Following this, it will be demonstrated that this new combination can add an extra step in the cascade of *click* reactions (*transclick* behaviour).

References:

Billiet S., Houck H.A., De Bruycker K., Winne J.M., Du Prez F.E., *To be submitted.*

Chapter VII

Towards a controlled cascade of triazolinedione-indole *transclick* reactions

VII.1 Introduction

As shown in the previous chapter, a novel conjugation strategy was developed, based on the unique reactivity of a triazolinedione (TAD) molecule, that combines the original-*click* properties with the possibility of a reversible bond-forming process. Due to its unique reactivity profile, TAD can act both as dienophile and enophile, making it susceptible to ultrafast Diels-Alder and Alder-*ene* reactions with diversely substituted (di)*enes* in an additive-free way. Based on the substitution pattern of the involved (di)*ene*, a range of reaction times can be obtained ranging from instantaneously to several hours (see chapter III). This kinetic tunability is however not the only opportunity that TAD molecules offer. By carefully choosing the complementary partner of TAD with a moderate reaction enthalpy, a reversible reaction can also be obtained. Chapter VI showed that when indoles, easily prepared basic heteroaromatic scaffolds, are reacted with TAD, the forward reaction pathway demonstrates *click* behaviour. However, when the resulting adduct is heated, the reversible nature starts to dominate, providing reshaping and healing properties. Additionally it was shown that the 'liberated' TAD moiety can be reacted (*in situ*) with another reactant (conjugated diene) in a subsequent *click* reaction. To generalize this remarkable, dynamic behaviour (exchanging of a *clickable* group between two differ-

ent molecules - indole and conjugated diene), a new concept called *transclick* reactions was introduced (see section VI.2.2).

In comparison to more well-known reversible reactions (e.g. furan-maleimide[1–4]), it became apparent that there was still room for additional research, more specifically concerning the tuning of the temperature window of the respective forward (bonding) and backward (debonding) reaction. The possibility to induce reversibility over such a broad temperature range has a profound effect on the material properties and eventual processing of the adducts and allows for 'ideal' temperature conditions to be tailored for the envisaged applications.[5]

Approaches that have been used in the past consist of changing the complementary partner (e.g. furan-maleimide => anthracene-maleimide[6–8]), replacing with other chemistries (e.g. cyclopentadiene-dithioesters[9–12]) or even making use of entropy.[13] Here we have chosen for the first strategy (changing the complementary partner), however with an important caveat. Instead of looking for a new molecule to partner with TAD, subtle changes on 1*H*-indoles molecules (with both electronic and steric effects) will be employed to tune the reversible behaviour. This has the advantage that the obtained knowledge of the original TAD-indole system can almost directly be implemented in this work, both for low molecular weight compounds as well as on polymer level.

In this chapter, a new TAD-indole combination is introduced, which has all of the *click* characteristics as the original system (see chapter VI) but possesses a different (lower) reversibility temperature. Additionally, it will be shown that this combination adds another level to the *transclick* reaction making it possible to purposely exchange a *clickable* group between three different moieties.

VII.2 Synthesis and exploration of novel indole substrates for TAD *transclick* reactions

For the original TAD-indole reactions 2-*tBu*-3-*iso*pentyl-1*H*-indole and 4-butyl-1,2,4-triazoline-3,5-dione were used as readily available model substrates. The basic indole structure, 2-*tBu*-1*H*-indole, is commercially available but not easy to obtain on large scale

(>50 g), therefore a more easily accessible (and cheaper) starting product, 2-phenyl-1*H*-indole (**94**), was used as building block in this chapter. Interestingly, indole **94** is also an approved additive for PVC materials (stabilizer), including food packaging plastic wraps, often used to more than 1% w/w.

VII.2.1 Synthesis of novel indole substrates with various substituents

Similar as in the case of 2-tBu-1*H*-indole (**88**), the phenyl group on the *C2* position of 2-phenyl-1*H*-indole (**94**) does not contain allylic hydrogens - therefore allowing the necessary selective imine adduct formation to take place (see section VI.2). If desired, a non-functional group can again be introduced on the *C3* position via a reductive alkylation (see Figure VII.1) to obtain a range of model components. Here, we chose to introduce the same non-functional *iso*-pentyl chain as in chapter VI, to yield 2-phenyl-3-*iso*pentyl-1*H*-indole (**95**). In this way, two similar indoles compounds (who only differ in the *C2* position) can be directly compared in function of the reaction kinetics and reversibility.

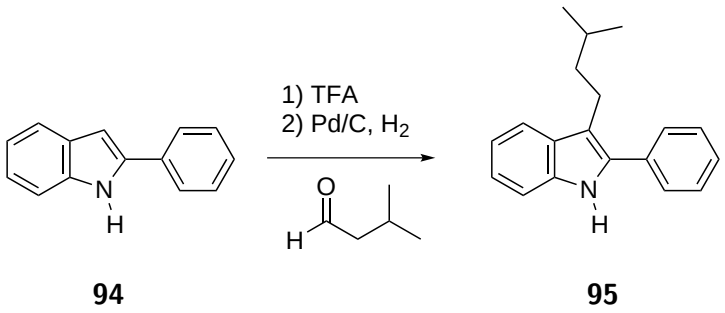
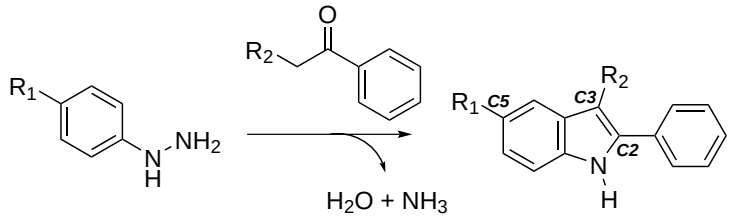


Figure VII.1: Introduction of a non-functional aliphatic group on the *C3* of 2-phenyl-1*H*-indole (**94**) to obtain 2-phenyl-3-*iso*pentyl-1*H*-indole (**95**).

An additional advantage of these 2-phenyl-1*H*-indoles (**94**) is its simple *one-step* synthesis from commercially available bulk building blocks (aromatic hydrazines and ketones) via the so-called *Fischer indole synthesis*.[14–16] In this way, a range of 2-phenyl-1*H*-indoles (**69**, **70**, **96** and **97**) can be readily prepared via a *one-step process*, see Figure VII.2, making it possible to introduce different side groups (both on the *C2*, *C3* and/or *C5*). In this chapter the *C2* position will always be a phenyl group (*vide infra*) but both *C3* and *C5* position alterations can be made by changing the phenylketone reaction partner. Non-functional model components were made by introducing an aliphatic (**69**) or aromatic

side chain (**70**) on the *C3* (coming from the starting ketone) and leaving the *C5* non-functionalized. A functional handle can be obtained by replacing phenylhydrazine by 4-hydrazino benzoic acid, giving a carboxylic acid at the *C5* position.



69	R_1 = H, R_2 = Me	**70**	R_1 = H, R_2 = Ph
96	R_1 = COOH, R_2 = Me	**97**	R_1 = COOH, R_2 = Ph

Figure VII.2: Fischer indole synthesis to yield 2-phenyl-1*H*-indoles with a different substitution pattern on the *C2*, *C3* and *C5* position (**69**, **70**, **96** and **97**).

VII.2.2 Influence of indole substitution pattern on reactivity with TAD

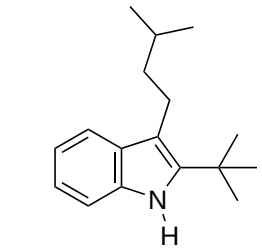
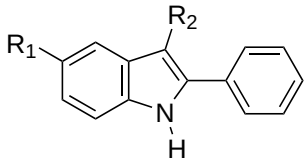
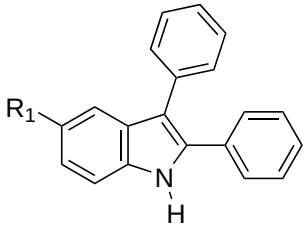
Having synthesized a range of different indole compounds, the reactivity with 4-butyl-1,2,4-triazoline-3,5-dione (BuTAD, (**31**)) was investigated. Prior to exploring the effect of the different substitution pattern on the reversibility temperature, the forward reaction was subjected to the same model studies (as discussed in section VI.2), in order to verify the (possible) *click* character.

First, the effect of replacing the *tert*-butyl group by a phenyl group was looked at in detail. Therefore, 2-phenyl-3-*iso*pentyl-1*H*-indole (**95**) was reacted with an equimolar amount of BuTAD (**31**) in DMSO-d_6 and checked via ^{1}H-NMR. The reaction went to completion in a few seconds (as judged by the disappearance of the red colour) and led to a single reaction product. The small but significant increase in reaction speed (reaction between 2-*tBu*-3-*iso*pentyl-1*H*-indole (**34**) and BuTAD went to completion in less than a minute) can most likely be ascribed to a combination of two factors: the decrease in sterical hindrance and the expanded conjugated system in the case of the phenyl substitution. Similar results were obtained when a shorter aliphatic chain (methyl instead of *iso*-pentyl) was introduced at the *C3*. This last result is important since 2-phenyl-3-methyl-1*H*-indole (**69**) can be obtained in high yield in one step (see VII.5.3.2). In a last experiment within this series

of aliphatic *C3* functionalized 2-phenyl-1*H*-indoles (**94**), the effect of a side chain on the *C5* was checked. Therefore, the same experiment was repeated with 2-phenyl-3-methyl-5-carboxy-1*H*-indole (**96**). Again no experimental differences were observed, leading to the conclusion that aliphatic *C3* functionalized 2-phenyl-1*H*-indoles (**94**) react very fast (order of seconds) with TAD independent of the length of the aliphatic chain on the *C3* or the presence of a functional handle at the *C5* position.

The same experiments were then performed on 2-phenyl-1*H*-indoles with an aromatic substituent on the *C3*. When 2,3-diphenyl-1*H*-indole (**70**) was reacted with BuTAD (**31**), the reaction went to completion but only after a much longer reaction time (3 hours instead of seconds). The increased sterical hindrance around the reaction site is expected to be the reason of this. Similar as with its aliphatic counterpart, the effect of *C5* functionalized was investigated (making use of 2,3-diphenyl-5-carboxy-1*H*-indole (**97**)) but again no experimental differences were observed.

Table VII.1: Reaction time of the reaction between the indoles studied in this doctoral work and TAD at room temperature in DMSO-d_6 (R_1 = H, COOH or COOMe, R_2 = Me or *i*-pentyl.

Indole	Reaction time
	<1 minute
	< 10 seconds
	3 hours

The *Fischer indole synthesis* provides a good alternative to synthesize a range of indoles components that can react in a *click-like* manner with TAD molecules. In comparison

with the original system (based on 2-*t*Bu-1*H*-indoles - **88**) having a phenyl *C2* substituent speeds up the reaction when an aliphatic *C3* chain is used. If however, an aromatic group is introduced at the *C3*, the reaction takes three hours. An additional advantage of this synthetic route is, the possibility of introducing a functional handle (at the *C5*) that is independent of the *C3* substitution while not altering the kinetics of the reaction itself (see Table VII.1).

VII.2.3 Study of the indole-to-diene *transclick* reaction of various TAD-indole adducts

Encouraged by the observed differences in reaction speed for the forward reaction, the newly synthesized TAD-indole adducts were subjected to reversibility tests. Since the reversibility temperature of 2-*t*Bu-3-isopentyl-1*H*-indole (**34**) was already determined in chapter VI, this value (90 °C) was taken as the reference for other indole components. Again the 5% threshold value, as introduced in section VI.2.2, will be used to define the reversibility temperature of TAD-indole systems. For ease of comparison the same model study with BuTAD, that was previously used (see section VI.2.2), was utilized for the 'new' indoles.

First, the influence of the phenyl group in the *C2* position was investigated. This was done by making use of 2-phenyl-3-*iso*pentyl-1*H*-indole (**95**) to determine if the phenyl group has influence of the reversibility temperature and 2-phenyl-3-methyl-1*H*-indole (**69**) to determine if the *Fischer indole synthesis* leads to similar results.

As an example the kinetic reversibility study for 2-phenyl-3-methyl-1*H*-indole (**69**) is depicted in Figure VII.3a. As before, two stock solutions (one of 2-phenyl-3-methyl-1*H*-indole and one of BuTAD) were prepared (in DMSO-d_6) and mixed. The reaction was followed via thin layer chromatography (TLC) and ^{1}H NMR spectroscopy. Via NMR, different signals could be followed to confirm a successful adduct formation at room temperature (see Figure VII.3b) in less than ten seconds. For instance, a urazole N-H signal (***H-4***) appears in the spectrum at the expense of the original N-H indole signal (***H-1***). Besides this, there is also a clear shift to higher field of the methyl protons (*C3* indole) in the formed adduct (***H-2*** respectively ***H-6***), changes in the aromatic range and a clear difference with the original BuTAD spectrum. The N-methylene signal (***H-3***) of the

butyl chain shifts clearly. This decrease in chemical shift is typical for BuTAD-*ene* reactions and can be explained by the higher electron density that the triazolinedione entity receives after the *ene*-reaction.

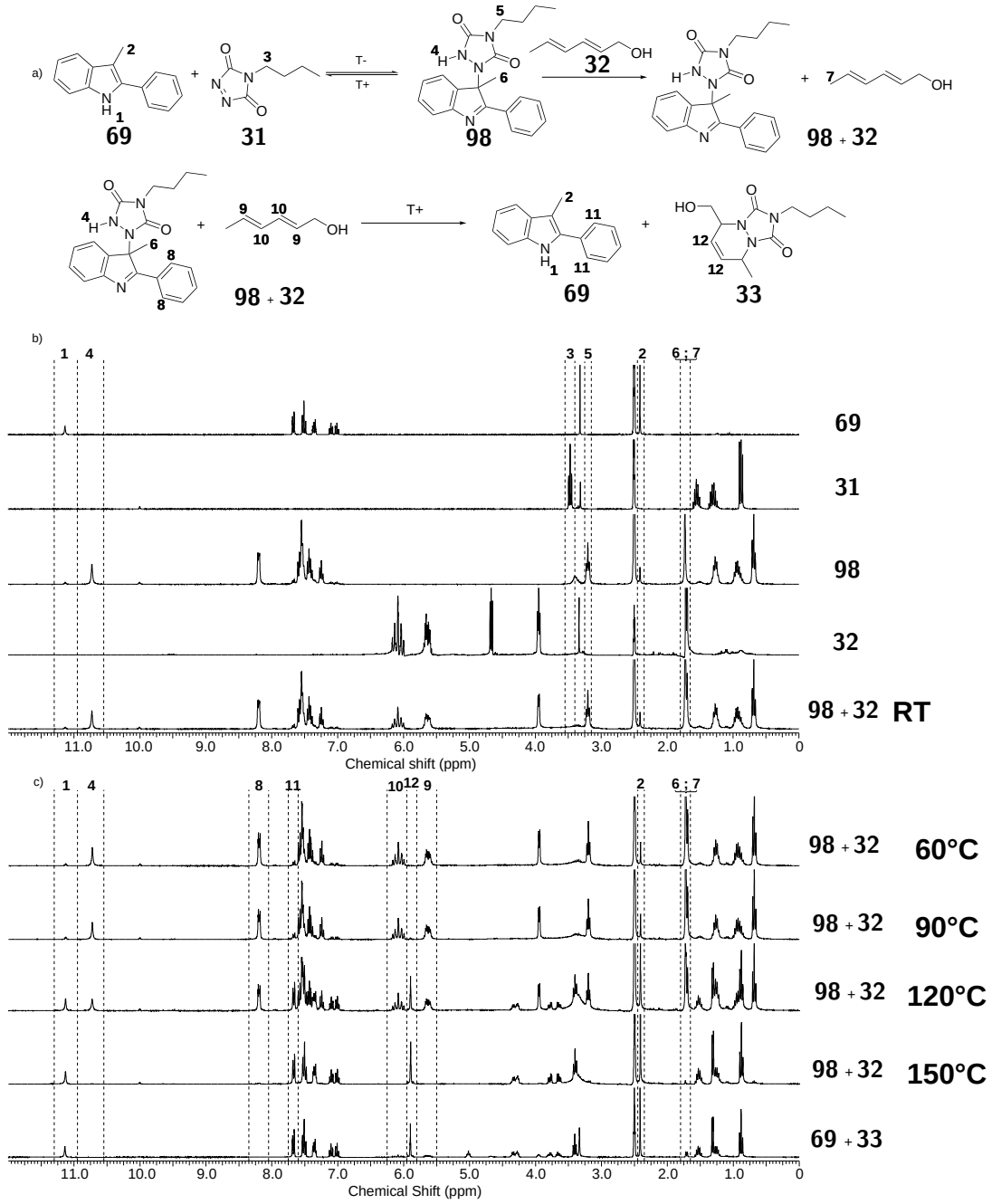


Figure VII.3: a) Reaction scheme for the kinetic reversibility study of indole (**69**) and BuTAD (**31**) , b) the resulting NMR spectra allow for the characterization of the starting mixture via the disappearance of the indole proton signals and the appearance of the adduct (**98**) signals and c) offline NMR analysis at different temperature can provide a reversibility profile for the reaction.

To capture (kinetically trap) and quantify the amount of BuTAD released during thermal treatments, a slight excess of *trans*,*trans*-2,4-hexadiene-1-ol (HDEO) (see **32** in Figure VII.3a) was added to the adduct at room temperature. This diene shows fast kinetics towards TAD components, resulting in the irreversible formation of a DA-adduct. When the TAD-indole-HDEO mixture is heated for 15 minutes, the triazolinedione is released and subsequently trapped in the HDEO-TAD adduct (**33**) via a *transclick* reaction (see chapter VI). The amount of observed HDEO-TAD adduct (**33**) can thus be related to the rate at which BuTAD is released from its indole adduct at the studied temperature (see Figure VII.3b).

Figure VII.3c shows the obtained spectra for the reversibility study, that illustrates how the adduct dissapears at higher temperatures (signals ***H-4*** and ***H-8***). At the same time an increase of the indole N-H signal **(*H-1*)** and decrease of the HDEO signals (***H-9*** and ***H-10***) is observed. This leads to the appearance of signals **(*H-12*)** of the newly formed DA adduct. Analysis of the spectra showed that no significant retro-reaction occurs in 15 minutes below 90 °C and heating to 150 °C results in the liberation of all indole moieties. Due to the fact that all signals in the spectrum of **98** + **32** during the reversibility study also appear in the spectrum of the reference **69** + **33**, the occurrence of significant side reactions at higher temperatures can be excluded.

To determine the reversibility profile, the ratio of the N-H signals (***H-1*** to ***H-4***) was used to calculate the fraction of newly formed adduct (due to overlap of the other signals). In contrast to the previous reversibility study this time a small excess of indole was used, so the only correct way to determine the reversibility was, in this example, the appearance of the new adduct and not the appearence of the free indole. Figure VII.4 shows the resulting reversibility profile – obtained via integration of the proton NMR-spectra – of 2-*t*Bu-3-*iso*pentyl-1*H*-indole (**34**) - the reference indole, 2-phenyl-3-*iso*pentyl-1*H*-indole (**95**) and 2-phenyl-3-methyl-1*H*-indole (**69**). Unfortunately, it can be seen that switching the side group on the *C2* starting component (from *t*Bu to phenyl) nor the size reduction of the aliphatic group on the *C3* position (from *iso*-pentyl to methyl) has a significant effect on the reversibility temperature profile and the reversibility temperature is almost identical for all the aliphatic *C3* functionalized indoles (see Figure VII.4). In light of Figure VI.1, we can conclude that the increase in forward reaction rate for indoles **69**

and **95** (relative to **34**) is most likely related to ground state destabilisation, and not to a lowering of the transition state energy relative to the TAD-indole adducts.

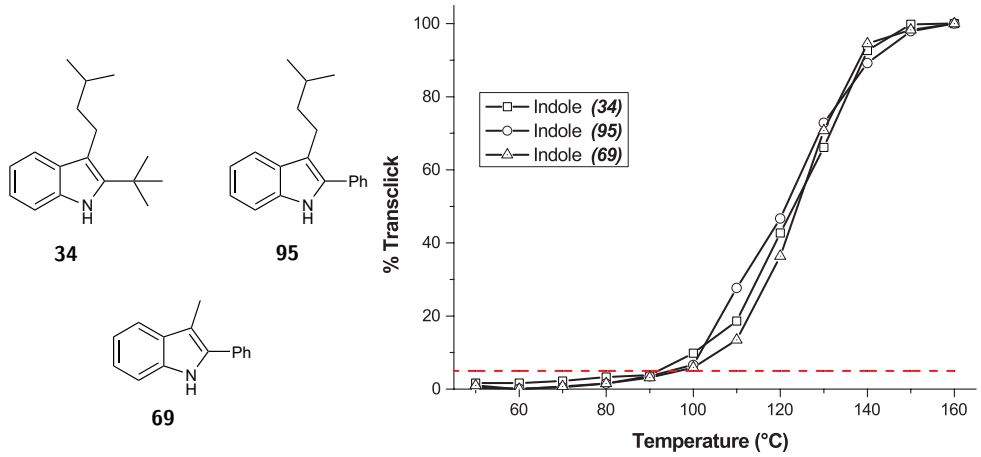


Figure VII.4: Involved indoles (**34**, **69** and **94**) and their respective reversibility curves.

After it was determined that switching the substitution pattern on the *C2* position does not change the reversibility temperature profile, the same tests were performed with a different substituent on the *C3* position. Figure VII.5 shows that the bulky (phenyl) substituent at the reaction site of to 2,3-diphenyl-1*H*-indole (**70**) has a profound effect on the reversibility profile of the *ene*-reaction. The retro-*ene* reaction starts at 70 °C (5%) and the TAD *transclick* reaction is completed in 15 minutes when treated at 120 °C. This is a very clear shift of 20 °C in comparison with the previously shown alkyl derivatives. This can be rationalised by the structure of the adduct **99** and starting indole (**70**). The phenyl group on the *C3* position selectively destabilizes the adduct through steric compression of bulky substituents around the newly formed C-N bond but not the starting component, making the reaction thermodynamically unfavourable. Significantly, this steric influence will be more pronounced in the adducts compared to a transition state structure in which the C-N bond formation is expected to be only partially completed. This combined effect can both explain the decreased forward reaction rate and the increased backward reaction rate (see Figure VI.1). It has to be noted that a phenyl group will also have an electronic effect besides a steric effect, thus the combination of both effects will play a role on the thermodynamic characteristics of the reaction. So far, unfortunately, all attempts to address this issue computationally have been remarkably unsuccessful.

As a last test, the effect of the introduced functional functional handle (carboxylic acid) was investigated. The changing electronic environment on the indole core that is created

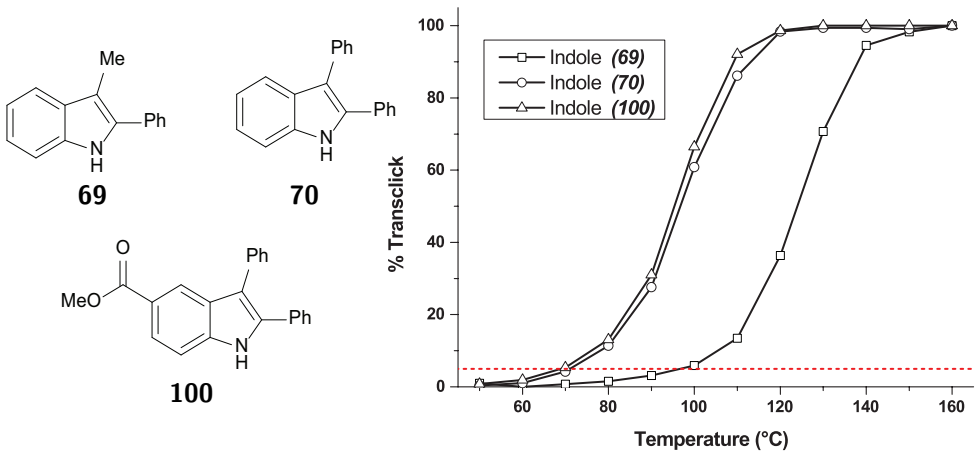


Figure VII.5: Indoles with aliphatic and aromatic substituents on the *C3* and their different reversibility behaviour. The red line on the curve represents the 5% limit where upon the reversibility temperature is determined for each indole.

by this electron-withdrawing substituent can also influence the reversiblity temperature profile. Therefore the indole precursor 2,3-diphenyl-5-carboxy-1*H*-indole (**97**) has to be submitted to the same reversibility test as before. As the carboxylic acid will be transformed, in most cases, into an ester functionality, the same is done for the model reaction. As can be seen in Figure VII.5, 2,3-diphenyl-5-carboxymethyl-1*H*-indole (**100**) shows the same reversibility curve as the non-functional indole (2,3-diphenyl-1*H*-indole, **70**). This clearly shows that introducing a functional handle on the *C5* position is a valuable route to obtain functionalized indole components, while it does not influence the overall reaction kinetics.

VII.3 Investigation of indole-to-indole TAD transclick reactions

After the successful identification of a new indole-TAD combination, that shows a markedly lower reversibility temperature, the exchange of a TAD between the two indoles was studied. This was accomplished at different temperatures based on the individual reversibility profiles (see Figure VII.5). Again a stock solution (in DMSO-d_6) was prepared, this time containing, in a 1:1 molar ratio, 2-phenyl-3-methyl-1*H*-indole (**69**) and the adduct (**99**) of BuTAD and 2,3-diphenyl-1*H*-indole. The adduct was obtained out of an equimolar reaction between BuTAD (**31**) and 2,3-diphenyl-1*H*-indole (**70**) and dried *in vacuo* (checked via NMR and LC-MS for purity). At room temperature, all BuTAD molecules are bonded

in the adduct (**99**) while 2-phenyl-3-methyl-1*H*-indole (**69**) is present as free component in the reaction mixture. Heating the sample for 15 minutes leads to the release of BuTAD that can immediately react with the more nucleophilic 2-phenyl-3-methyl-1*H*-indole (see Figure VII.6a).

Although the newly formed adduct also has a pronounced reversibility in the investigated temperature range, the involved NMR spectra show that after cooling only this adduct is observed. This is obvious from the spectra shown in Figure VII.6c where the proton resonance of the new adduct and 2-phenyl-3-methyl-1*H*-indole (**69**) are gradually replaced by those of the new adduct (**98**) and 2,3-diphenyl-1*H*-indole (**70**) with increasing temperature. The same observation is valid for the aromatic signals ***H-2*** and ***H-6***, that each represent two protons in their respective TAD-*ene* adducts. By normalizing the integration of these signals, it becomes possible to follow the progress of the exchange reaction as a function of the temperature (see Figure VII.6b). This shows that the exchange reaction actually goes to full conversion (>99%) if the reaction mixture is heated at 120 °C for 15 minutes.

When the experiment is compared with the original reversibility curve of 2,3-diphenyl-1*H*-indole wherein TAD is trapped by a diene scavenger (red line overlay in Figure VII.6b), it becomes apparent that these curves are closely related. This shows that the *rate determining step* of this TAD transfer reaction is the retro-*ene*-reaction of BuTAD and (**70**) in both cases (indole or diene trapping partners). If HDEO is added to this reaction mixture at room temperature, a simple addition spectrum is obtained. The new signals are all coming from the diene while the urazole signals **(*H-5*)** are broadened due to hydrogen bonding. This is not the case for the indole N-H signal **(*H-4*)**, due to the acidic behaviour of the urazole protons.

Eventually it is possible, via a quantitiave retro-*ene* reaction at 150 °C, to kinetically trap all the liberated BuTAD in the HDEO-adduct (**33**) and simultaneously expel 2-phenyl-3-methyl-1*H*-indole selectively. The choice for this last temperature comes from the kinetic reversibility study of 2-phenyl-3-methyl-1*H*-indole to have a fast exchange.

After demonstrating the consecutive *transclick* reactions on low molecular weight components, the next step was to transform this to macromolecular context. In the previous

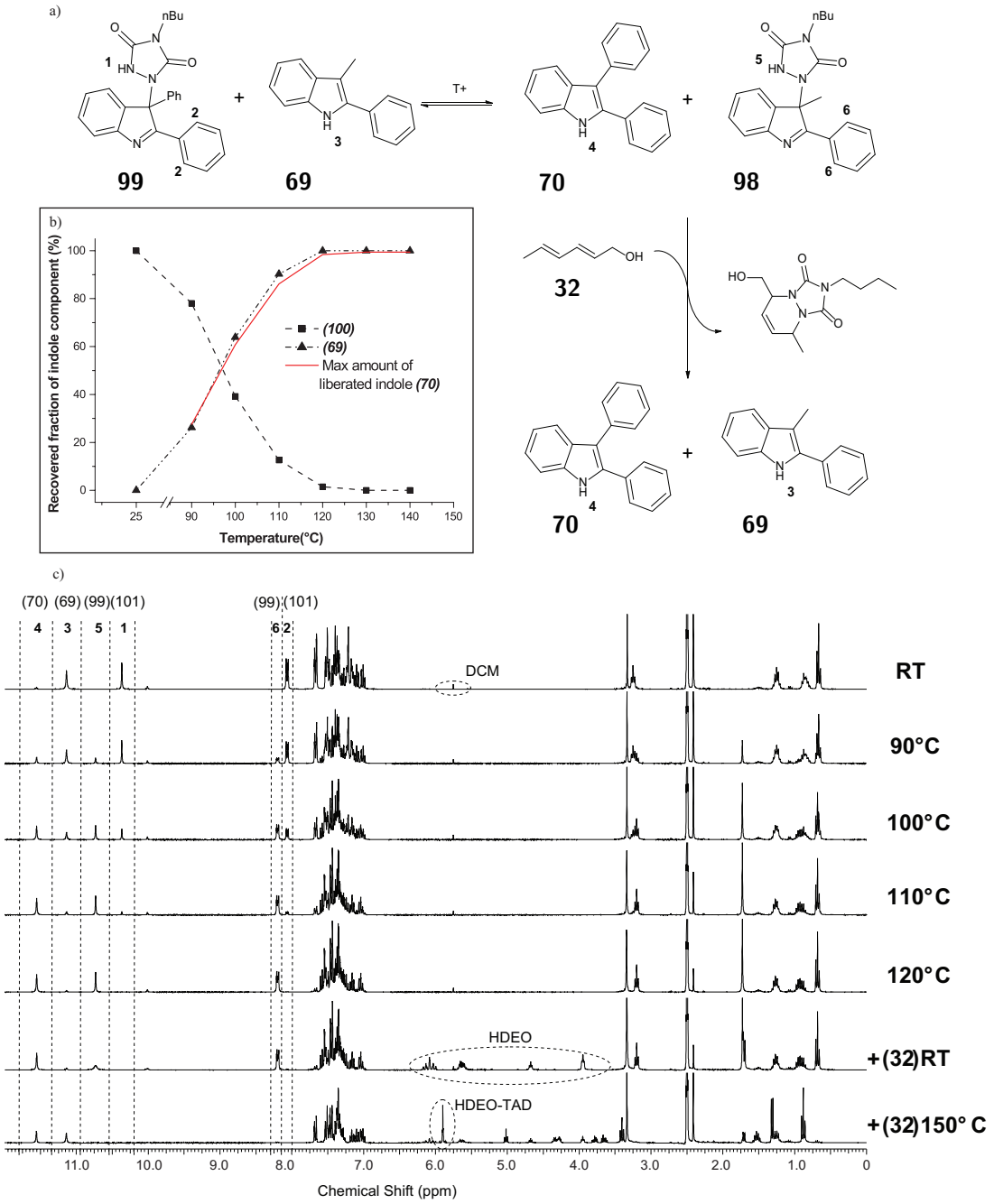


Figure VII.6: a) Selective exchange of the TAD-indole adducts when a reaction mixture of *ene*-adduct (**99**) and indole (**69**) is heated, b) graphical representation of the exchange reaction in function of the temperature where the decrease of adduct (**99**) in the reaction mixture is compensated with the increase of adduct (**98**). The red line represents the maximal fraction of transfer, as deducted from the kinetic reversibility study of indole (**70**) and c) ^{1}H-NMR spectra after heating show the exchange of the indole N-H and urazole N-H signals (15 minutes of heating at 150 °C in the presence of HDEO (**32**) releases in the end indole (**69**).

chapter, the successful combination of controlled radical polymerization (CRP) and TAD-indole chemistry was shown by implementing *Cu(0) mediated polymerization*. In order to broaden the scope of TAD chemistry it was envisaged to implement another form of CRP,

reversible addition-fragmentation chain-transfer (RAFT) polymerization. An additional advantage of RAFT polymerization is the high tolerance of this technique towards acidic protons.[17] Since urazole moieties possess a very acidic proton (pKa = 4,7)[18], this was expected to lead to a better control of the living character of the polymerization. However due to time constraints it was not possible to include this data in this doctoral work.

VII.4 Conclusions and perspectives

In this chapter a 'new' *TAD-indole* combination that showed a different reversibility temperature than the one discussed in chapter V, was introduced. This led to the conclusion that TAD chemistry can provide, besides a *kinetic tuneability* for the forward reaction rates, also a tunable reversibility profile.

First, the reaction between TAD and a different starting 1*H*-indole (2-phenyl-1*H*-indole) was studied. This indole had the same desired protection of the *C2* position and can be obtained in a more straightforward way than the previous indole building blocks. Following this, an indole derivative (2,3-diphenyl-1*H*-indole) was prepared which contained a more sterically hindered *C3* position. The goal was to selectively destabilize the TAD-indole adduct in respect to the starting components and push the adduct to relatively higher energy levels compared to the starting materials which would lead to a lower reversiblity temperature. This approach was successful, lowering the reversibility temperature to 70 °C (difference of 20 °C).

In a second step, the previously introduced *transclick* concept was expanded with this TAD-indole combination. This led to the successful *cascade* of three different *click* reactions. For this a monofunctional TAD molecule was selectively transferred from the first TAD-indole adduct (based on 2,3-diphenyl-1*H*-indole) to the second TAD-indole combination (based on 2,3-methyl-1*H*-indole) and in a final step to the DA adduct. To the best of our knowledge, this is the first example of such a selective and clear transfer via *click* reactions.

In a last step this knowledge should have been brought to macromolecular level, however due to lack of time this was not possible within the doctoral work. It will however be continued in the near future within our own research group.

This chapter introduced a new TAD-indole combination with a lower reversibility temperature and an orthogonal reactivity profile. The obtained insights and results lead to new projects further exploring TAD-indole chemistry to end up with a full control over reversibility temperature. In the ideal case this would lead to a suitable TAD-indole combination for almost every specific application that desires a reversible behaviour between room temperature and 150 °C.

VII.5 Experimental section

VII.5.1 Materials

Acetic acid (glacial), 4-hydrazinobenzoic acid (97%), *trans*,*trans*-2,4-hexadien-1-ol (>97%), 3-methylbutyraldehyde (97%), palladium (5% on activated carbon), 2-phenylacetophenone (97%), phenylhydrazine (97%), potassium hydroxide (reagent grade, 90%, flakes), propiophenone (99%), sulfuric acid (99%) and trifluoro acetic acid (99%) were purchased from Sigma-Aldrich. Ethyl carbazate (97%), hexamethylene diisocyanate (>98%) and hydrochloric acid (4N, in dioxane) from Acros Chemicals, ammonium chloride(99%), potassium carbonate (99%) and sodium carbonate (99%) from Roth, 2-phenyl-1*H*-indole (>98%) from TCI, hydrochloric acid (36%) from Chem-Lab and magnesium sulphate (anhydrous) from Boom. All solvents (Sigma-Aldrich) and products were used without any pre-treatment or purification.

VII.5.2 Characterization

Nuclear Magnetic Resonance (NMR) ^{1}H-spectra were recorded with a Bruker Avance 300 (300 MHz) FT-NMR spectrometer in $CDCl_3$ (Eurisotop) or DMSO-d_6 solution at room temperature. Chemical shifts are presented in parts per million (δ) relative to $CHCl_3$ (7.26 ppm for ^{1}H-NMR) and DMSO (2.50 ppm for ^{1}H-NMR) as an internal standard. The resonance multiplicities are descried as [br. (broad)] s (singlet), d (doublet), t (triplet), q (quadruplet), quin (quintuplet), sext (sextuplet) or m (multiplet).

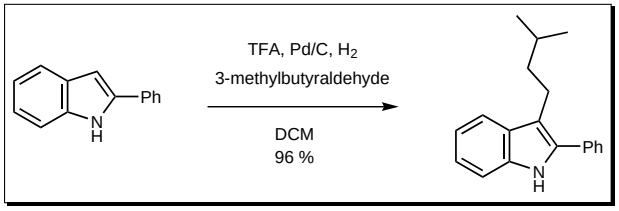
Liquid Chromatography - Mass Spectrometry (LC-MS) analyses were performed on an Agilent Technologies 1100 series LC/MSD system with a diode array detector (DAD) and a single quad MS. Analytical reversed phase HPLC-analyses were performed with a Phenomex Luna C18 (2) column (5 μm, 250 mm $\times$ 4.6 mm) and a solvent gradient (0-100 % acetonitrile in H_2O in 15 min). The eluted compounds were analyzed via UV detection (214 nm).

VII.5.3 Synthesis

The synthesis of the following compounds can be found in previous chapters:

- 4-Butyl-1,2,4-triazoline-3,5-dione (BuTAD, **31**) - see III.8.3.1
- 1,4-Diazabicyclo[2.2.2]octane bromide complex (DABCO-Br) - see III.8.3.2
- 2-*tert*-Butyl-3-isopentyl-1*H*-indole (**34**)- see III.8.3.3
- 2-Phenyl-3-methyl-1*H*-indole (**69**) - see III.8.3.11
- 2,3-Diphenyl-1*H*-indole (**70**) - see III.8.3.12

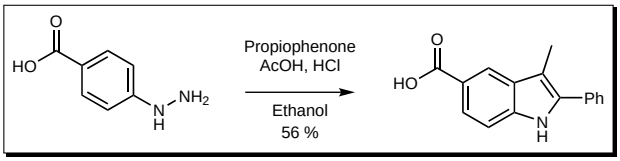
VII.5.3.1 2-phenyl-3-*iso*pentyl-1*H*-indole (94)



In a two neck flask of 100 mL, a mixture of trifluoro acetic acid (0.6 mL, 7.76 mmol, 1.5 equivalent) and palladium (5% on activated carbon) in dichloromethane (12 mL) was made. The resulting mixture was placed under hydrogen atmosphere and stirred in an ice bath. To this a mixture of 2-phenyl-1*H*-indole (1.0 g, 5.17 mmol, 1 equivalent) and 3-methylbutyraldehyde (0.61 mL, 5.69 mmol, 1.1 equivalent) in dichloromethane (18 mL) was added dropwise. This solution was stirred vigorously (in a water bath) for five hours, regularly flushing with hydrogen gas. With the help of TLC (hexane:ethyl acetate 9:1) the reaction was followed until completion. The mixture was filtered over celite and washed with saturated aqueous sodium carbonate solution (30 mL). The organic phases are dried over magnesium sulfate and concentrated *in vacuo* to obtain pure 2-phenyl-3-isopentyl-1*H*-indole as a brown oil (1.34 g – 96%).

^{1}H-NMR (300 MHz, DMSO−$\mathbf{d_6}$): δ (ppm) = 0.92 (d, 6 H, CH_3), 1.55 (m, 2 H, i−Pr−CH_2), 2.84 (t, 2 H, CH_2−CH$_2$−i−Pr), 7.00 (t, 1 H, NH−C−CH=CH−CH), 7.10 (t, 1 H, NH−C−CH=CH), 7.36 (m, 2 H, ArH), 7.50 (m, 3 H, ArH), 7.62 (m, 2 H, CH$_2$−C=C−C=CH), 11.11 (s, 1 H, NH).

VII.5.3.2 2-phenyl-3-methyl-5-carboxy-1*H*-indole (96)



In a two neck flask (250 mL) a mixture of concentrated acetic acid (50 mL) and 15 mL concentrated hydrochloric acid (15 mL) is added to 4-hydrazino benzoic acid (1.00 g, 6.57 mmol, 1.0 equivalent) and propiophenone (0.87 mL, 6.57 mmol, 1.0 equivalent). The mixture was refluxed (at 120 °C) for three hours and then cooled down to room temperature. By slow addition of water (60 mL), the title compound precipitates out of solution. The pure product was obtained by filtration and thoroughly dried overnight in a vacuum oven to yield a green powder (0.92 g, 56%).

Bruto formula: $C_{17}H_{25}N$.

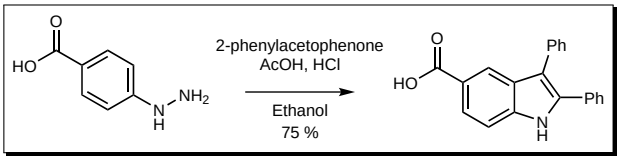
MW.: 243.39 g/mol.

MS (m/z for [MH]+): experimental: 252.1.

^{1}H-NMR (300 MHz, DMSO−d_6): δ (ppm) = 2.24 (s, 3 H, CH_3), 7.35-7,43 (m, 2 H, ArH), 7.53 (t, 2 H, ArH), 7.65-7,71 (m, 2 H, ArH), 7.74 (d, 1 H, ArH),8.21 (s, 1 H, HOOC−C−CH−C), 11.54 (s, 1 H, NH), 12.40 (s, 1 H, COOH).

^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) = 9.62 (CH_3), 108.06 (C), 110.70 (CH), 121.07 (CH), 121.13 (C), 122.85 (CH), 127.40 (CH), 127.62 (CH), 128.76 (CH), 128.88 (C), 132.45 (C), 135.27 (C), 138.38 (C), 168.37 (C)

VII.5.3.3 2,3-diphenyl-5-carboxy-1*H*-indole (97)

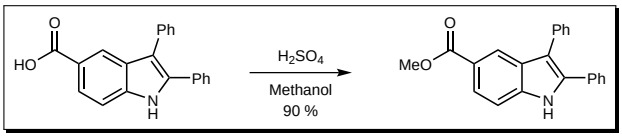


In a two neck flask (500 mL) a mixture of concentrated acetic acid (200 mL) and concentrated hydrochloric acid (60 mL) was added to 4-hydrazino benzoic acid (3.00 g, 19.7 mmol, 1 equivalent) and 2-phenylacetophenone (3.87 g, 19.7 mmol, 1 equivalent). The mixture was refluxed (at 120 °C) for three hours and then cooled down to room temperature. By slow addition of water (250 mL), 2,3-diphenyl-5-carboxy-1H-indole crystallizes

out of solution. The pure product was obtained by filtration and dried overnight in a vacuum oven (4.62 g, 75%).

^{1}H-NMR (300 MHz, DMSO$-$d$_6$): δ (ppm) = 7.28-7,54 (m, 11 H, Ar*H*), 7.80 (d, 1 H, Ar*H*), 8.13 (s, 1 H, HOOC$-$C$-$C*H*$-$C), 11.94 (s, 1 H, N*H*), 12.48 (s, 1 H, COO*H*).

VII.5.3.4 2,3-diphenyl-5-carboxymethyl-1*H*-indole (100)



In a two neck flask (25 mL) a mixture of 2,3-diphenyl-5-carboxy-1*H*-indole ((**97**, 0.3 g, 1.19 mmol) and concentrated sulfuric acid (1 mL) in methanol (10 mL) was stirred at room temperature until the carboxylic acid disappeared (TLC, ethyl acetate:heptane 1:9). After extraction with ethyl acetate (40 mL), the solution was washed with an saturated aqueous sodium carbonate solution (2 x 10 mL) and dried over magnesium sulphate. By concentrating *in vacuo* pure 2,3-diphenyl-5-carboxymethyl-1*H*-indole is obtained (0.283 g, 90

^{1}H-NMR (300 MHz, DMSO$-$d$_6$): δ (ppm) = 3.82 (s, 3 H, C*H*$_3$$-$C(O)$-$O), 7.29-7,51 (m, 10 H, Ar*H*), 7.53 (d, 1 H, Ar*H*), 7.81 (d, 1 H, Ar*H*), 8.12 (s, 1 H, MeOOC$-$C$-$C*H*$-$C), 11.99 (s, 1 H, N*H*).

VII.5.3.5 Reversibility studies

A general procedure to determine the reversibility profile is depicted here. Two stock solutions are prepared, the first one of BuTAD (**31**, 75 mg, 0.5 mmol, 1equivalent) in DMSO-d_6 (6 mL) and the second one of the desired test indole (0.5 mmol, 1 equivalent) also in Dimethylsulfoxide-d$_6$ (DMSO-d_6, 6 mL). The two solution were mixed and stirred until the reaction went to full conversion (checked via TLC). To determine the composition of the reaction mixture at elevated temperature *trans*,*trans*-2,4-hexadien-1-ol (HDEO) (52 mg, 0.55 mmol, 1.1 equivalent) in DMSO-d_6 (1.5 mL) was added. Via ^{1}H NMR the obtained adduct (before and after addition of HDEO) was characterized and compared with the original spectra. The resulting solution was divided into numerous NMR samples and studied in the desired temperature range (50 $-$160 °C). The different NMR samples

were placed in an oil bath for 15 minutes at the desired temperature. Via ^{1}H NMR the composition of the mixture was determined. In order to determine the percentage of *transclick* all spectra were normalized.

- ***ene* adduct of 2-tert-butyl-3-isopentyl-1*H*-indole (34) and BuTAD**

  ^{1}H-NMR (300 MHz, DMSO−d_6): δ (ppm) = 0.73 (m, 6 H, CH(CH_3)$_2$ + CH_3−CH$_2$−CH$_2$−CH$_2$), 0.89 (m, 2 H, CH$_3$−CH_2−CH$_2$−CH$_2$), 1.03 (m, 2 H, CH$_3$−CH$_2$−CH_2−CH$_2$), 1.32 (s, 9 H, C(CH_3)$_3$), 1.71 (dd, 3 H, CH_2−CH(CH$_3$)$_2$), 2.29 (m, 2 H, NH−N−C−CH_2), 3.24 (t, 2 H, CH_2−CH$_2$−CH$_2$−CH$_3$), 6.75-7,5 (band, 4 H, ArH), 10.62 (s, 1 H, NH).

- **DA adduct (33) of *Trans,trans*-2,4-hexadien-1-ol and BuTAD**

  ^{1}H-NMR (300 MHz, DMSO−d_6): δ (ppm) = 0.88 (t, 3 H, CH_3−CH$_2$−CH$_2$−CH$_2$), 1.27 (sext, 2 H, CH$_3$−CH_2−CH$_2$−CH$_2$), 1.33 (d, 3 H, CH_3−CH), 1.52 (quin, 2 H, CH$_3$−CH$_2$−CH_2−CH$_2$), 3.39 (t, 2 H, CH$_3$−CH$_2$−CH$_2$−CH_2), 3.65 (br d, 1 H, HCH−OH), 3.77 (br d, 1 H, HCH−OH), 4.30 (m, 2 H, CH−N−N−CH), 5.00 (br s, 1 H, HO−CH$_2$), 5.91 (s, 2 H, CH=CH).

- ***ene* adduct of 2-phenyl-3-isopentyl-1*H*-indole (95) and BuTAD**

  ^{1}H-NMR (300 MHz, DMSO−d_6): δ (ppm) = 0.65 (t, 3 H, CH_3−CH$_2$−CH$_2$−CH$_2$), 0.78 (sext, 2 H, CH$_3$−CH_2−CH$_2$−CH$_2$), 0.92 (d, 6 H, (CH_3)$_2$−CH), 1.25 (quin, 2 H, CH$_3$−CH$_2$−CH_2−CH$_2$), 1.55 (m, 2 H, i−Pr−CH_2), 1.70 (m, 1 H, (CH$_3$)$_2$−CH), 1.84 (t, 2 H, i−Pr−CH$_2$−CH_2), 3.28 (t, 2 H, CH$_3$−CH$_2$−CH_2−CH_2), 7.2-7,45 (band, 7 H, ArH), 8.11 (d, 2 H, CH$_3$−C−C(=N)−C−CH), 10.50 (s, 1 H, NH).

- ***ene* adduct (98) of 2-phenyl-3-methyl-1*H*-indole and BuTAD**

  ^{1}H-NMR (300 MHz, DMSO−d_6): δ (ppm) = 0.70 (t, 3 H, CH_3−CH$_2$−CH$_2$−CH$_2$), 0.92 (sext, 2 H, CH$_3$−CH_2−CH$_2$−CH$_2$), 1.25 (quin, 2 H, CH$_3$−CH$_2$−CH_2−CH$_2$), 1.70 (s, 3 H, CH_3−C), 3.21 (t, 2 H, CH$_3$−CH$_2$−CH_2−CH_2), 7.2-7,42 (band, 7 H, ArH), 8.20 (d, 2 H, CH$_3$−C−C(=N)−C−CH), 10.60 (s, 1 H, NH).

- ***ene* adduct of 2-phenyl-3-methyl-5-carboxy-1*H*-indole (97) and BuTAD**

  ^{1}H-NMR (300 MHz, **DMSO$-$d$_6$**): δ (ppm) = 0.68 (t, 3 H, C$H_3$$-CH_2$$-CH_2$$-CH_2$), 0.90 (sext, 2 H, CH$_3$$-CH_2$$-CH_2$$-CH_2$), 1.23 (quin, 2 H, CH$_3$$-CH_2$$-CH_2$$-CH_2$), 1.68 (s, 3 H, C$H_3$$-$C), 3.20 (t, 2 H, CH$_3$$-CH_2$$-CH_2$$-CH_2$), 7.11-7,62 (band, 6 H, ArH), 8.20 (d, 2 H, CH$_3$$-C-$C(=N)$-C-CH$), 10.60 (s, 1 H, N$H$),12.49 (s, 1 H, COO$H$).

- ***ene* adduct (99) of 2,3-diphenyl-1*H*-indole and BuTAD**

  ^{1}H-NMR (300 MHz, **DMSO$-$d$_6$**): δ (ppm) = 0.65 (t, 3 H, C$H_3$$-CH_2$$-CH_2$$-CH_2$), 0.78 (sext, 2 H, CH$_3$$-CH_2$$-CH_2$$-CH_2$), 1.25 (quin, 2 H, CH$_3$$-CH_2$$-CH_2$$-CH_2$), 3.26 (t, 2 H, CH$_3$$-CH_2$$-CH_2$$-CH_2$), 7.2-7,42 (band, 12 H, ArH), 8.09 (d, 2 H, CH$_3$$-C-$C(=N)$-C-CH$), 10.30 (s, 1 H, N$H$).

- ***ene* adduct of 2,3-diphenyl-5-carboxy-1*H*-indole (97) and BuTAD**

  ^{1}H-NMR (300 MHz, **DMSO$-$d$_6$**): δ (ppm) = 0.67 (t, 3 H, C$H_3$$-CH_2$$-CH_2$$-CH_2$), 0.78 (sext, 2 H, CH$_3$$-CH_2$$-CH_2$$-CH_2$), 1.27 (quin, 2 H, CH$_3$$-CH_2$$-CH_2$$-CH_2$), 3.21 (t, 2 H, CH$_3$$-CH_2$$-CH_2$$-CH_2$), 7.09-7,52 (band, 11 H, ArH), 8.08 (d, 2 H, CH$_3$$-C-$C(=N)$-C-CH$), 10.32 (s, 1 H, N$H$), 12.19 (br s, 1 H, COO$H$).

- ***ene* adduct of 2,3-diphenyl-5-carboxymethyl-1*H*-indole (100) and BuTAD**

  ^{1}H-NMR (300 MHz, **DMSO$-$d$_6$**): δ (ppm) = 0.67 (t, 3 H, C$H_3$$-CH_2$$-CH_2$$-CH_2$), 0.78 (sext, 2 H, CH$_3$$-CH_2$$-CH_2$$-CH_2$), 1.27 (quin, 2 H, CH$_3$$-CH_2$$-CH_2$$-CH_2$), 3.21 (t, 2 H, CH$_3$$-CH_2$$-CH_2$$-CH_2$), 3.80 (s, 3 H, C$H_3$$-$C(O)$-$O) 7.09-7,52 (band, 11 H, ArH), 8.08 (d, 2 H, CH$_3$$-C-$C(=N)$-C-CH$), 10.32 (s, 1 H, N$H$), 12.19 (br s, 1 H, COO$H$).

VII.5.3.6 *Transclick* model reaction

Two stock solutions were prepared, the first one of BuTAD (**31**, 85 mg, 0.5 mmol, 1.1 equivalent) in dichloromethane (6 mL) and the second one of 2,3-diphenyl-1*H*-indole (**70**, 135 mg, 0.5 mmol, 1 equivalent) also in dichlorometane (6 mL). The two solutions were mixed and stirred until the reaction went to full conversion (checked via TLC). The obtained adduct was dried overnight *in vacuo* (removing the volatile excess of BuTAD) to give the pure adduct. The obtained adduct (100 mg) was mixed with 2-phenyl-3-methyl-1*H*-indole (**69**, 49 mg, 0.24 mmol, 1 equivalent) and dissolved in DMSO-d_6 (6 mL). The mixture was heated in an oil bath at 120 °C for 15 minutes after which via ^{1}H NMR the obtained reaction mixture was characterized and compared with the original spectra (before heating). In order to check the last step of the *transclick* reaction, HDEO (**32**, 26 mg, 0.264 mmol, 1.1 equivalent) was added and the mixture was heated at 150 °C for 15 minutes. Again via ^{1}H NMR the composition was determined.

VII.6 Bibliography

[1] J. Craven, *US3435003-A* **1969**, Cross–linked thermally reversible polymers produced from condensation polymers with pendant furan groups cross–linked with maleimides.

[2] X. Chen, M. A. Dam, K. Ono, A. Mal, H. Shen, S. R. Nutt, K. Sheran, F. Wudl, *Science* **2002**, *295*, 1698–1702.

[3] X. Chen, F. Wudl, A. K. Mal, H. Shen, S. R. Nutt, *Macromolecules* **2003**, *36*, 1802–1807.

[4] X. Chen, *Phd Dissertation (University of California)* **2003**, Novel polymers with thermally controlled covalent cross–linking.

[5] P. Espeel, F. E. Du Prez, *Macromolecules* **2015**, *48*, 2–14.

[6] J. R. Jones, C. L. Liotta, D. M. Collard, D. A. Schiraldi, *Macromolecules* **1999**, *32*, 5786–5792.

[7] J. A. Syrett, G. Mantovani, W. R. S. Barton, D. Price, D. M. Haddleton, *Polymer Chemistry* **2010**, *1*, 102–106.

[8] N. Yoshie, S. Saito, N. Oya, *Polymer* **2011**, *52*, 6074–6079.

[9] A. J. Inglis, S. Sinnwell, T. P. Davis, C. Barner-Kowollik, M. H. Stenzel, *Macromolecules* **2008**, *41*, 4120–4126.

[10] A. J. Inglis, M. H. Stenzel, C. Barner-Kowollik, *Macromolecular Rapid Communications* **2009**, *30*, 1792–1798.

[11] A. Inglis, S. Sinnwell, M. Stenzel, C. Barner-Kowollik, *Angewandte Chemie International Edition* **2009**, *48*, 2411–2414.

[12] M. Glassner, G. Delaittre, M. Kaupp, J. P. Blinco, C. Barner-Kowollik, *Journal of the American Chemical Society* **2012**, *134*, 7274–7277.

[13] N. K. Guimard, J. Ho, J. Brandt, C. Y. Lin, M. Namazian, J. O. Mueller, K. K. Oehlenschlaeger, S. Hilf, A. Lederer, F. G. Schmidt, M. L. Coote, C. Barner-Kowollik, *Chemical Science* **2013**, *4*, 2752–2759.

[14] E. Fischer, F. Jourdan, *Berichte der deutschen chemischen Gesellschaft* **1883**, *16*, 2241–2245.

[15] E. Fischer, O. Hess, *Berichte der deutschen chemischen Gesellschaft* **1884**, *17*, 559–568.

[16] S. Müller, M. J. Webber, B. List, *Journal of the American Chemical Society* **2011**, *133*, 18534–18537.

[17] C. Barner-Kowollik, *Handbook of RAFT Polymerization*, **2008**.

[18] S. Radl in *1,2,4-Triazoline-3,5-Diones*, *Vol. 67*, Elsevier, San Diego, **1997**.

Chapter VIII

General conclusions and perspectives

VIII.1 General Conclusions

The aim of this doctoral work was to develop a versatile *click* chemistry platform, based on triazolinediones (TADs) as synthetic tools, that offers a wide *tunability* both in terms of kinetics and reversibility. This *tunable reactivity* can have an enormous impact on many applications and might be an overlooked characteristic for *click* and other efficient reactions. Although the use of TAD moieties in organic (and polymer) synthesis had been hinted at[1,2] in preliminary studies and some highly specific applications, examples of TAD *click-like* reactions were not investigated in detail before this work (see Chapter II). In this work the focus lies on the *click* properties of the TAD *Diels-Alder* (DA) and *Alder-ene* (AE) reactions (see Figure VIII.1).

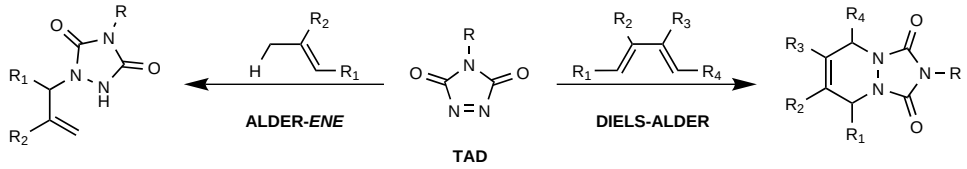


Figure VIII.1: The Diels-Alder (right) and the Alder-*ene* reaction (left) involving a triazolinedione (TAD) molecule.

Since TADs are considered uncommon or even exotic reagents by most chemists, this work started with a literature survey (chapter II).[3] In a first step, focus is placed on the synthesis of these components. This is followed by a short overview of the remarkable reactivity of TAD molecules, the focal point being the reactions that play an important role in this work. After this an overview is provided of the use of triazolinediones in

polymer science. In a last part TAD molecules are described in the context of *click-like* chemistry. In chapter III an overview of relevant characteristics (orthogonality, introduction of functional handles, kinetics, stability and toxicity) led to the conclusion that the newly introduced platform shows robustness and the potential to be generally utilized.

In chapter IV, both Diels-Alder and Alder-*ene* reactions involving TAD molecules were investigated for their *click-like* behaviour. For both reactivity modes, model experiments on low molecular weight compounds were performed. It was demonstrated that TAD DA and AE reactions indeed closely match *click* chemistry ideals. Following this, examples of macromolecular functionalization were also achieved using TAD DA *click* reactions. For this reaction, rate constants of up to 160 000 $M^{-1}s^{-1}$ are typical (see section III.4)[4], two orders of magnitude higher then the fastest known *click* reaction[5], thus the *ultrafast* formation of block copolymers was studied (see Figure VIII.2). A final application of the DA TAD *click* reaction in polymer chemistry is the synthesis of networks.[6]

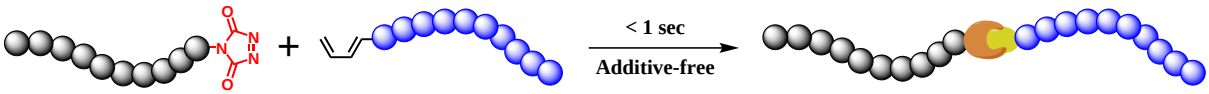


Figure VIII.2: Ultrafast block copolymer formation between a TAD-functionalized and a diene-functionalized polymer.

In an additional example of the power and versatility of TAD chemistry, crude vegetable oils were employed for the additive-free preparation of polymer materials from chemically unrefined and renewable resources (chapter V). Most of the building blocks of plant oils, fatty acids, contain double bonds that can undergo AE *click* reactions with TAD moieties. To study the various reactions that can occur, model studies on the most common natural fatty acids were described. The obtained knowledge was then implemented in the chemical crosslinking of crude (commercially available) plant oils. This led to a wide variety of polymer networks (see Figure VIII.3) with varying physical properties, that could be related to the starting plant oil and/or used crosslinker.[7]

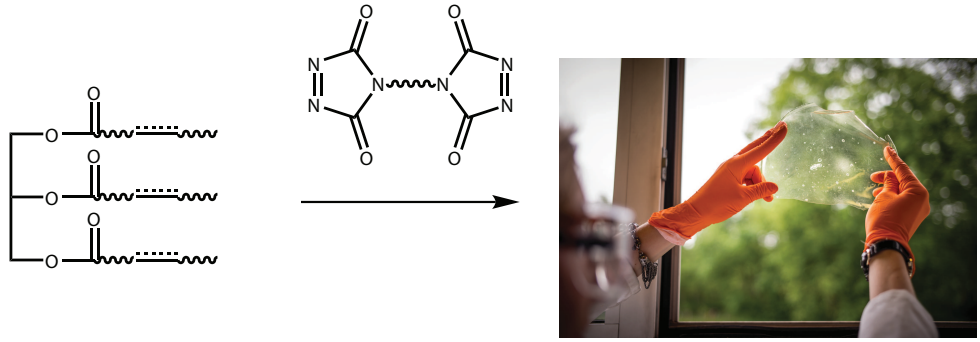


Figure VIII.3: Formation of plant oil based materials (in this case a *plant foil*) based on reaction between commercially available plant oil and a bifunctional TAD molecule.

After demonstrating the *click* character of various TAD-based reactions and considering the ability to tune the kinetic behaviour over a wide range (see chapter III.4) by simply changing the complementary partner, a very interesting question remained - at least from the point of view of polymer chemistry applications - was then addressed in this work. '*Can TAD chemistry provide reversible click reactions?*' Besides kinetic tunability of the forward reaction, the possibility to control the reaction rate of the reverse reaction is of great interest as well. In this light, chapter VI investigated the reaction between triazolinediones and 1*H*-indoles (see Figure VIII.4) as potential *click* reactions that can be reversed at elevated temperatures, but still behaves as a normal *click* reaction should at ambient temperatures.[6]

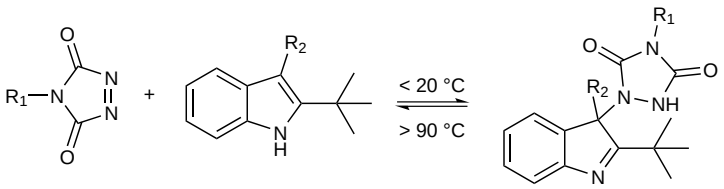


Figure VIII.4: Reversible *click* reaction between a TAD molecule and a 1*H*-indole.

When the *click* characteristics of the AE coupling between TAD and 1*H*-indoles were demonstrated, a series of kinetic experiments were performed to investigate the required temperature for the retro AE reaction in a semi-quantitative way. In order to monitor the 'retro-*click*' reaction between indoles and TAD compounds, it proved necessary to add a different reaction partner for the liberated TAD compounds. This is because TAD compounds apparently are too reactive to lead to any discernable concentration build-up in free form, even when the TAD-indole reaction becomes dynamic. To our surprise this 'transfer of TAD reagents' appeared to be a very clean process that, moreover, was not

described in literature yet. Therefore the term *transclick* reaction was introduced and it was defined that any reaction that is a covalent linking process that can be triggered (in this case by temperature) to form a new bond with another reaction partner and at the same time release one of the original binding partners, in which each bond-forming step meets all the typical *click* characteristics.[6]

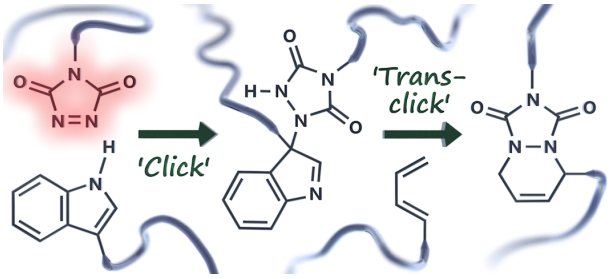


Figure VIII.5: *Transclick* reaction on polymer level: a TAD-functionalized polymer reacts in a first *click* reaction with an 1*H*-indole-functionalized polymer. After heating the block copolymer in presence of a diene, the TAD-polymer is transferred (in a *click*-way) to the DA adduct and the starting indole-polymer is obtained.

This *transclick* concept was demonstrated in macromolecular functionalization by first synthesizing a block copolymer (based on TAD-indole chemistry) that was subsequently heated - in the presence of a trapping (diene) molecule - to release one of the original polymer chains, while the other one is now covalently bonded to another partner (see Figure VIII.5). In a last stage, a variety of TAD-indole-based polymer networks were synthesized that showed dynamic behaviour that could be used for recycling, reshaping...

Inspired by the *tunable kinetic behaviour* of TAD reactions, the question arose if further fine-tuning could be achieved for the reversible TAD-indole reaction itself. In chapter VII, different TAD-indole combinations were investigated, some of which showed a significantly lower reversibility temperature (20 °C less). Following this, the newly introduced *transclick* concept was expanded with this TAD-indole combination. This led to the design and implementation of a *cascade* of three different *click* reactions. In this cascade, a monofunctional TAD molecule was selectively transferred from the first TAD-indole adduct (based on 2,3-phenyl-1*H*-indole) to the second TAD-indole combination (based on 2-phenyl-3-methyl-1*H*-indole) and in a final step to the DA adduct (see Figure VIII.6). To the best of our knowledge, this is a the first example of such a selective and clear transfer via *click* reactions. It was our desire to show this new cascade of *click* reaction on macromolecular level, however due to lack of time this was not possible within the

doctoral work.

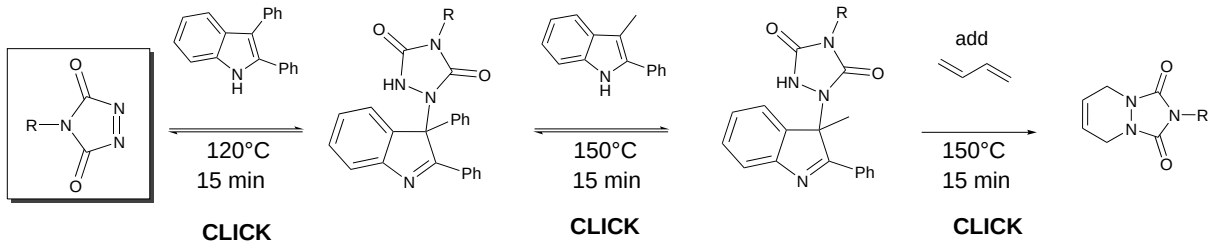


Figure VIII.6: 'Indole-indole-diene' *Transclick* reaction: a simplified representation of the *click* transfer of a TAD molecule on three levels.

In this doctoral thesis, the core chemistry of a newly developed *click* chemistry platform based on the unique reactivity of triazolinediones, is described. Besides being yet another good reaction for *click* chemistry, TAD chemistry also provides remarkable reaction kinetics that are not present in previous *click* chemistries. This can lead to a choice in kinetic behaviour (a difference of 8 orders of magnitude can be obtained). Additionally also reversible reactions can be obtained via TAD chemistry. In this respect, a new concept (a *transclick* reaction) was introduced that shows a truly unique 'transfer' behaviour for TAD molecules.

VIII.2 Future perspectives

During the course of a doctoral work, a whole range of new ideas and projects come along. In this respect, this PhD thesis is not an exception. As a already mentioned throughout this work, several (new) PhD projects (six up to date) and two industrial projects, involving TAD chemistry, were started up in the course of these last four years. Besides this, around 20 collaborations with (inter)national groups, in a vast area of research domains, were set up. However, despite the numerous research that is ongoing at this moment, there are still some important topics that need to be addressed in the near future.

Due to the limited commercial availability of TAD components (see chapter III), there still remains a huge need to synthesize them. This doctoral work tried to lead the way by investigating general and upscalable synthetic routes for TAD molecules (something which is also taken up by other PCR group members). In the case of crosslinkers and non-functional molecules, this was readily achieved. For TADs containing functional handles, this was unfortunately not possible for all relevant functional groups (diols,

carboxylic acids, thiols, azides...) Although some TAD components containing these functional handles were explored, the known examples remained small scale and specific to a certain project. For this reason, we are convinced that a crucial step towards a broad adaptation of TAD chemistry, is the continued synthetic effort towards these functional TAD molecules.

Once more functional TAD molecules are available, the step to more complex applications (and material properties) can be made. In polymer science, efficient (*click*) reactions are used for the synthesis of complex architectures (dendrimers, hyperbranched polmyers...) and cyclic polymers. By designing these structures, the *click* character of TAD chemistry can be emphasized. An additional advantage is the *tunable behaviour* (both kinetic and thermodynamic) that TAD chemistry can provide, which can lead to ´new' possibilities. In regard to the *transclick* applications, the programmable and highly reliable transfer of TAD reagents between up to three different substrates opens up unique possibilities. Indeed, affinity-based transfer of compounds/chemicals from one specific site to another, perhaps mediated by an in-between modulator, evoke 'chemical signaling' pathways which are usually only observed in protein-mediated cell biology systems.

A last aspect that will be crucial for the further development of TAD *click* chemistries, closely related to the thermodynamic tunability, is the temperature limit of TAD chemistry. As discussed in section III.5.1, TAD compounds cannot be heated above 150 °C, in order to avoid decomposition. Since this is a inherent characteristic of a TAD molecule, this cannot be overlooked when TAD chemistry is introduced in a 'new' field and/or application. As long as the temperature of the (reversible) TAD chemistry is kept below 150 °C no issues should arise, however this already excludes a large range of classical chemical processes.

It can be concluded that there remains a lot of challenges for TAD chemistry, especially on synthetic level. However, it is our strong belief that the advantages and new characteristics that the TAD *click* (and *transclick*) platform brings are versatile enough to rapidly find its way into the standard toolbox of many research disciplines.

VIII.3 Bibliography

[1] S. Radl in *1,2,4-Triazoline-3,5-Diones*, *Vol. 67*, Elsevier, San Diego, **1997**.

[2] G. B. Butler, *Industrial and Engineering Chemistry Product Research and Development.* **1980**, *19*, 512–528.

[3] K. De Bruycker, S. Billiet, H. Houck, S. Chattopadhyay, J. Winne, F. Du Prez, *Chemical Reviews* **2015**.

[4] V. D. Kiselev, D. A. Kornilov, I. I. Lekomtseva, A. I. Konovalov, *International Journal of Chemical Kinetics* **2015**, *47*, 289–301.

[5] M. L. Blackman, M. Royzen, J. M. Fox, *Journal of the American Chemical Society* **2008**, *130*, 13518–13519.

[6] S. Billiet, K. De Bruycker, F. Driessen, H. Goossens, V. Van Speybroeck, J. M. Winne, F. E. Du Prez, *Nature Chemistry* **2014**, *6*, 815–821.

[7] O. Türünç, S. Billiet, K. De Bruycker, S. Ouardad, J. Winne, F. E. Du Prez, *European Polymer Journal* **2015**, *65*, 286–297.

Chapter IX

Nederlandstalige samenvatting

IX.1 Algemene introductie

Kolb, Finn en Sharpless initieerden in 2001 een nieuw onderzoeksgebied dat de chemische wereld grondig zou beïnvloeden. Ze publiceerden namelijk een review artikel dat een nieuwe strategie voor organische synthese beschreef, met verwijzing naar de term *klik-chemie*. Deze naam beschrijft eigenlijk een set van regels, die tegemoet komen aan de behoeften van het hedendaagse onderzoek en hebben niet alleen voor een grote verschuiving gezorgd in het synthetisch, methodologisch onderzoek, maar vooral ook in de toepasbaarheid van synthesemethoden in allerlei niet-chemische toepassingen. Hoewel de originele kijk van de auteurs vooral gericht was op geneesmiddelenontwikkeling, namen verschillende andere chemische richtingen, zoals bijvoorbeeld de polymeerwetenschappen, dit concept zeer snel over.[1]

De natuur heeft het ongelooflijke talent om op een quasi perfecte manier reversibele carbonyl chemie te controleren, iets wat een syntheticus tot op heden jammer genoeg nog niet beheerst. Daarom verschuift de focus van *klikchemie* weg van deze klassieke organische opbouwreacties naar zeer efficiënte (kinetisch gecontroleerde) reacties die betrouwbaar en selectief zijn. Sharpless en medewerkers definieerden dat *klik*-reacties moesten voldoen aan een reeks van strikte criteria - '*modulair, algemeen toepasbaar, hoge opbrengst, enkele onschadelijke bijproducten produceren (die eenvoudig kunnen verwijderd worden), stereospecifiek zijn, eenvoudig uit te voeren en het gebruik van geen (of eenvoudig te verwijderen) solventen*'- om te voldoen aan het label van *klikchemie.*[2] In het ideale geval zijn de gebruikte startproducten dan ook nog vlot verkrijgbaar.

Aan al deze eigenschappen voldoen is geen eenvoudige taak, toch zijn er al verschillende reacties die geïdentificeerd zijn als *klikreacties* - de meest gekende zijnde de *koper(I)-gekatalyseerde azide-alkyn cycloadditie* (CuAAC).[3,4] Geleidelijk claimden steeds meer chemische platformen de titel van *klikreactie*, zoals de nucleofiele ringopening reacties (epoxides, aziridines, aziridinium ionen), non-aldol carbonyl-chemieën (vormen van urea, oximes en hydrazonen), addities aan koolstof-koolstof dubbele bindingen en cyclo-addities. Wanneer het voorlopig beperkte aantal industriële applicaties aangaande *klikchemie* (Invitrogen™, Allozyne™, Aileron™, Integrated Diagnostics™, Baseclick™, Active Motif Chromeon™, Cyandye™...) gedetailleerd bestudeerd worden, wordt het duidelijk dat ze tot op heden het meest geïmplementeerd is in twee domeinen, namelijk de bio- en materiaalwetenschappen. Een voorbeeld van de eerste categorie kan gevonden worden in het efficiënt toevoegen van een label aan een biomolecule, terwijl dit doctoraatsonderzoek een voorbeeld probeert te zijn van de ontwikkeling van *klikchemie* in de materiaalwetenschappen.

IX.1.1 Het gebruik van *klikchemie* in polymeerwetenschappen

Ondanks het feit dat Sharpless en medewerkers het meeste nut voorzien hadden voor *klikchemie* in applicaties aangaande de synthese van biologisch actieve moleculen, is de impact die dit werk voortgebracht heeft, misschien wel het grootste in de polymeerwetenschappen.[5] Bij het ontwikkelen en synthetiseren van gefunctionaliseerde polymeerstructuren, kunnen de efficiënte *klikreacties* immers een grote rol spelen, dit in combinatie met het ontbreken van nevenproducten en daarbij horende eenvoudigere opzuivering. Uiteindelijk heeft dit zelfs geleid tot de synthese van nog niet eerder bereikte structuren.[6] Samen met het succes van *klikchemie* (en alle bijhorende aandacht), kwam er ook een enorme stijging van het aantal publicaties daarover. Jammer genoeg werd de term dan ook al snel misbruikt, net om deze extra aandacht te verkrijgen. In een poging om dit te beperken, werd door enkele wetenschappers, op initiatief van de eigen onderzoeksgroep, een uitbreiding (of eigenlijk een beperking) van de oorspronkelijke definitie geïntroduceerd en dit specifiek gericht op het gebruik in polymeerwetenschappen.[7] Figuur IX.1 toont de originele karakteristieken - gedefinieerd door Sharpless - in combinatie met de nieuwe polymeereigenschappen. Twee zeer belangrijke eigenschappen - grote schaal opzuivering en equimolariteit - werden op deze wijze geïntroduceerd. De 'traditionele' opzuiveringsmeth-

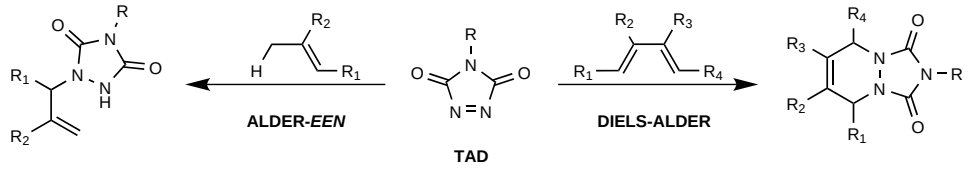
Figuur IX.1: Voorwaarden voor *klikreacties* (blauw: originele voorgesteld door Sharpless; groen en blauw-groen: aangepaste voorwaarden bij gebruik van één of meerdere polymeren en rood: de extra criteria voor aanpasbaar gedrag).

oden (zoals destillatie) zijn niet mogelijk wanneer er gewerkt wordt met polymeren. Daarbij komt nog dat de schaal waarop gewerkt wordt met polymeren dit nog extra bemoeilijkt. Equimolariteit is misschien wel het meeste cruciale in een polymeercontext, gebrek hieraan zou immers een zuivere polymeer-polymeer koppeling onmogelijk maken.[8–10] Sinds het introduceren van deze 'striktere definitie' is de zoektocht naar geschikte combinaties van reagentia in polymeercontext druk aangaande.[11,12] Tot aan de start van dit doctoraatsproject was nog geen volledig universeel kliksysteem beschreven. In het ideale geval zou zo een systeem niet enkel moeten voldoen aan alle *klikchemie* karakteristieken maar ook de keuze geven tussen irreversibele en reversibele verbindingen. Hoewel dit op het eerste zicht misschien contra-intuïtief lijkt met het *klikconcept*, is er, zeker in het polymeeronderzoek, een enorme vraag naar dynamische of reversibele systemen voor bijvoorbeeld zelfherstellende materialen, recyclage of het herwerken van netwerken. Tot op vandaag is er nog geen universeel *klikchemie* platform beschreven dat voldoet aan de reeds beschreven eigenschappen in combinatie met een aanpasbaar gedrag (reversibel tot irreversibel) in een nuttig temperatuursgebied. In dit doctoraatsonderzoek wordt het gebruik van triazolinedionen naar voor geschoven als middel om een dergelijk universeel

klikplatform te benaderen.

IX.1.2 Triazolinedion gebaseerde *klikchemie*

1,2,4-Triazoline-3,5-dion (TAD) gebaseerde molecule zijn heterocyclische componenten met een azo-verbinding, gebonden aan twee carbonylgroepen.[13] Deze minder gekende moleculen bezitten een uitzonderlijke reactiviteit die het best kan vergeleken worden met singlet zuurstof, waarbij *ultra-snelle* Diels-Alder en Alder-*een* reacties de voorkeur hebben (zie Figuur IX.2).



Figuur IX.2: Diels-Alder (rechts) en Alder-*een* reactie (links) met een triazolinedion (TAD) molecule.

Deze Diels-Alder en Alder-*een* reacties zijn het meest gekend voor hun orthogonaliteit en (misschien het belangrijkste) hun reversibliteit - en dit ondanks het feit dat veel systemen, zoals de populaire Diels-Alder reactie van furan en maleïmides, niet voldoen aan alle *klik-karakteristieken.*[14–16] TAD componenten daarentegen bieden een ruim assortiment aan selectieve reacties die een zeer hoge opbrengst opleveren onder equimolaire condities bij lage temperaturen (<20 °C) en zonder dat het toevoegen van additieven of een katalysator nodig is.[17] Een bijkomend voordeel van deze reacties is de intense rode kleur van de TAD-componenten, die voor een visueel feedback systeem zorgt, aangezien de meeste reactieproducten kleurloos zijn.[18]

Ondanks het feit dat het gebruik van TAD-moleculen in *klik-achtige* reacties reeds beschreven is[19], ontbrak bij de start van dit onderzoek een volledige studie die alle *klik-eigenschappen* van zowel Diels-Alder en Alder-*een* TAD reacties beschrijft. Dit werk is bedoeld als een introductie op het gebruik van triazolinedionen in de ontwikkeling van een universeel *klikplatform* dat zeer moduleerbaar is en dit zowel op gebied van kinetiek als reversibiliteit.

IX.2 Overzicht van het proefschrift

Hoofdstuk II geeft een overzicht van de theoretische achtergrond van triazolinedionen (TADs). Om de structuur van dit hoofdstuk meer overzichtelijk te maken, is de bespreking van de synthese opgedeeld in ten eerste de synthetische strategieën voor urazolen (de precursor van triazolinedionen) en ten tweede de daaropvolgende oxidatie van urazolen om TAD moleculen te bekomen. Dit wordt gevolgd door een kort overzicht van de uitzonderlijke reactiviteit van TAD moleculen, met de nadruk op reacties die een belangrijke rol spelen in dit werk. Hierna wordt een beeld geschetst van het gebruik van triazolinedionen in een polymeercontext. Als laatste onderdeel wordt een samenvatting gegeven van de implementatie van TAD-moleculen in de context van *klikchemie*.

Hoofdstuk III beschrijft alle relevante chemische en andere eigenschappen van triazolinedionen. Deze gedetailleerde discussie van relevante karakteristieken (orthogonaliteit, kinetiek, introductie van functionele groepen, stabiliteit en toxiciteit) is cruciaal om TAD-chemie te begrijpen en zo efficiënt mogelijk te implementeren.

Hoofdstuk IV onderzoekt het mogelijke *klikgedrag* van TAD chemie en dit meer bepaald in het geval van Diels-Alder en Alder-*een* reacties. Hiervoor worden modelstudies uitgevoerd en ondersteund door theoretische berekeningen. Hierop volgend worden voorbeelden van deze chemie besproken in een macromoleculaire context.

Hoofdstuk V beschrijft de additief-vrije synthese van polymeermaterialen door het combineren van TAD-chemie met commercieel beschikbare plantaardige oliën. In een eerste stap worden modelstudies uitgevoerd met de meeste voorkomende vetzuren. De opgedane kennis kan dan geïmplementeerd worden bij de synthese van verschillende polymeernetwerken door de chemische vernetting van ruwe plantaardige oliën met een reeks gesynthetiseerde, bifunctionele TAD moleculen. In een laatste stap worden de ontwikkelde materialen dan volledig gekarakteriseerd.

Hoofdstuk VI toont het reversibele luik van het *klikplatform*, gebaseerd op de unieke reactiviteit tussen triazolinedionen en indolen. Deze moleculen kunnen een *klikreactie* ondergaan maar wanneer het gevormde adduct opgewarmd wordt, toont dit reversibiliteit. Hierna werd een nieuw concept *transklik* geïntroduceerd. Dit nieuw concept beschrijft

een trapsgewijze opeenvolging van *klikreacties* door het gebruik van de reeds vermelde reversibiliteit. Dit wordt zowel op modelstudies als op polymeerniveau uitgebreid bestudeerd.

Hoofdstuk VII voegt een extra (temperatuurs)niveau toe aan het reversibele *klikconcept*. Door het aanpassen van de substituenten van de gebruikte indoolcomponenten, wordt een lagere reversibiliteitstemperatuur bereikt. Dit levert een extra niveau op voor het nieuwe *transklik* platform en daarbij horend een extra mogelijkheid voor de opeenvolging van de TAD *klikreacties*. Een gelijkaardige strategie als in de vorige hoofdstukken wordt gebruikt om dit te onderbouwen op laag moleculaire componenten. Helaas was het, door tijdsgbrek, niet mogelijk om een studie op polymeerniveau bij te voegen.

Hoofdstuk VIII geeft een algemene conclusie van het beschreven doctoraatswerk en licht enkele toekomstplannen toe voor het nieuw ontwikkelde *klikchemie* platform.

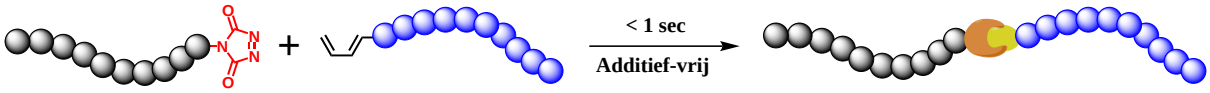
IX.3 Besluiten voor de verschillende hoofdstukken

Het doel van dit doctoraatswerk was de ontwikkeling van een universeel *klikchemie* platform - gebaseerd op het gebruik van triazolinedionen (TADs) - dat volledig kan afgesteld worden in functie van de kinetiek en de thermodynamische eigenschappen. Dit aanpasbaar gedrag kan een enorme impact hebben op vele applicaties en is misschien wel een 'vergeten' eigenschap in de context van *klikchemie* en andere efficiënte reacties. Ondanks het feit dat TAD-moleculen reeds besproken zijn in organisch (en polymeer) syntheses, is het gebruik van deze moleculen in een *klikcontext* nog vrij zeldzaam (zie hoofdstuk II).[17,20] In dit werk lag de focus op de *klikeigenschappen* van de TAD Diels-Alder (DA) en Alder-*een* (AE) reacties.

Omdat TADs relatief onbekend zijn voor het brede publiek, was de start van dit doctoraatsonderzoek een overzicht te voorzien van de relevante literatuur (hoofdstuk II).[21] Eerst werd de focus gelegd op de synthese van TAD componenten, gevolgd door een kort overzicht van de reactiviteit (de nadruk lag hier op reacties die van belang waren voor dit onderzoek). Hierna werd het gebruik van TAD-moleculen in polymeertoepassingen besproken. Als laatste werd dan het gebruik van TAD in *klikchemie* nader toegelicht. In hoofdstuk III lag de nadruk op het bespreken van relevante chemische en andere

eigenschappen van TAD-moleculen, dit door een combinatie van literatuur en eigen onderzoek. Op deze manier werd aangetoond dat het nieuw geïntroduceerde platform zeer robuust is en het potentieel heeft om snel gebruik te worden.

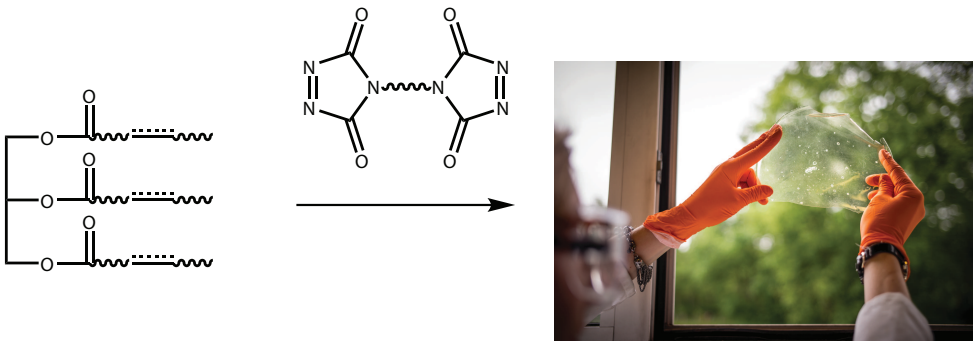
In hoofdstuk IV werd aangetoond dat zowel de Diels-Alder als de Alder-*een* reactie met TAD-moleculen *klikeigenschappen* vertonen. Dit werd aangetoond aan de hand van modelstudies en ondersteund door theoretische berekeningen. Hierna werden voorbeelden getoond van polymeerconjugaties met behulp van de *ultra-snelle* TAD DA chemie. Voor deze reactie kunnen snelheidconstanten tot $160\,000\,M^{-1}s^{-1}$ bekomen worden (zie sectie III.4), twee grootte-ordes groter dan de snelst geraporteerde *klikchemie*[22] en werd de *ultra-snelle* vorming van blockcopolymeren bestudeerd (see Figuur IX.3). Als finaal voorbeeld van de TAD DA *klikreactie* in polymeerchemie werd de synthese van netwerken voorgesteld.[23]



Figuur IX.3: *Ultra-snelle* vorming van een blockcopolymeer door reactie tussen een TAD-gefunctionaliseerd en een dieen-gefunctionaliseerd polymeer.

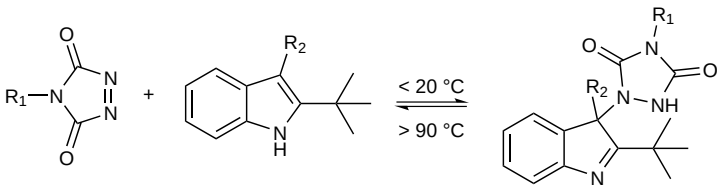
Hierop volgend werd een extra voorbeeld van de kracht van TAD chemie beschreven, waarbij commercieel beschikbare plantaardige oliën werden gebruikt voor de synthese van polymeermaterialen op basis van hernieuwbare grondstoffen (hoofdstuk V). De meeste bouwstenen van plantoliën en vetzuren bevatten dubbele bindingen en kunnen een AE *klikreactie* ondergaan met TAD moleculen. Om de verschillende reacties die kunnen plaatsvinden te onderzoeken, werden modelstudies op de meeste voorkomende vetzuren uitgevoerd. Deze nieuwe kennis werd onmiddellijk geïmplementeerd in het chemisch vernetten van ruwe (commercieel verkrijgbare) plantoliën. Op deze manier kon een brede waaier aan poylmeernetwerken bekomen (zie Figuur IX.4) met uiteenlopende fysische eigenschappen (afhankelijk van de gebruikte olie en/of vernetter).[24]

Na het demonsteren van de *klikeigenschappen* van verschillende TAD-gebaseerde reacties en rekening houdend met de mogelijkheid om het kinetisch gedrag te contoleren, door een eenvoudige verandering van complementaire partner, werd een interessante vraag (zeker vanuit het standpunt van de polymeerwetenschappen) beantwoord in dit doctoraatswerk. '*Kan TAD chemie met haar unieke kinetische eigenschappen ook voor thermodynamisch*



Figuur IX.4: Vorming van een materiaal op basis van een plantaardige olie (in dit geval een *plantfolie* ontstaan uit de reactie tussen een commercieel beschikbare plant olie en bisfunctionele TAD molecule.

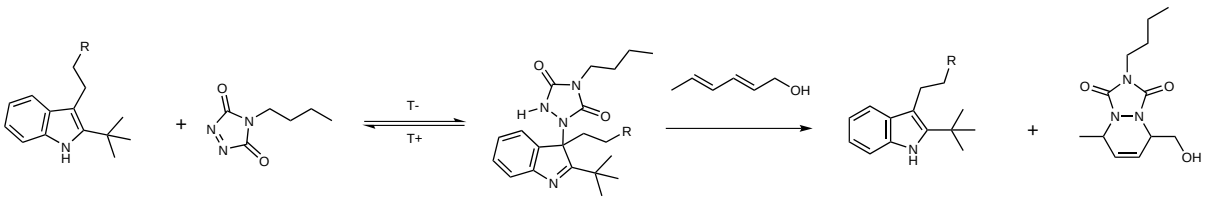
reversibele reacties zorgen?' Naast het controleren van het kinetisch gedrag van de voorwaartse reactie, is ook de mogelijkheid tot controle over de reactiesnelheid van de omgekeerde reactie van groot belang. In kader hiervan werd in hoofdstuk VI de reactie tussen triazolinedionen en 1*H*-indolen (zie Figuur IX.5) bestudeerd als mogelijke *klikreactie* (bij kamertemperatuur) die omgekeerd kan worden bij verhoogde temperatuur.[23]



Figuur IX.5: Reversibele *klikreactie* tussen een TAD molecule en een 1*H*-indool.

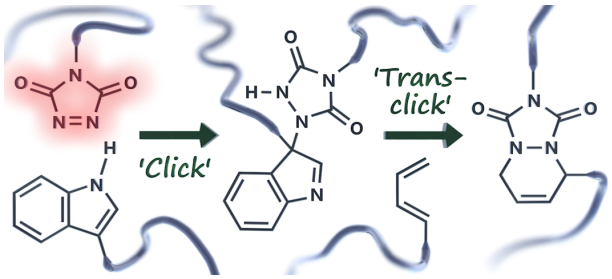
Nadat de *klikeigenschappen* van de AE reactie tussen TAD en 1*H*-indoles waren gedemonstreerd, werd een reeks van kinetische experimenten uitgevoerd om de benodigde temperatuur voor de terugwaartse reactie te onderzoeken op een semi-kwantitatieve wijze. Om de terugwaartse *klikreactie* tussen TAD en indolen te volgen, was het noodzakelijk om een additionele partner voor de vrijgestelde TAD-moleculen te introduceren. De reden hiervoor is dat TAD componenten bij hoge temperatuur te reactief zijn om tot een meetbare concentratie te leiden, zelfs wanneer de TAD-indool adduct dynamisch wordt. Enigzins tot onze verbazing, bleek deze 'transfer van TAD reagentia' heel zuiver te verlopen, meer nog, dit gedrag was nog nergens beschreven in de literatuur voor een *klik*reactie. Om deze reden werd de term *transklik* reactie geïntroduceerd en werd dit gedefinieerd als *elke reactie die een covalent bindingsproces is, die geactiveerd kan worden (in ons geval door temperatuur) om een nieuwe binding aan te gaan met een andere*

reactiepartner én op hetzelfde moment één van de originele partners terug vrijstelt. Hierbij verloopt elke reactie volgens de typische klikeigenschappen (zie Figuur IX.6).[23]



Figuur IX.6: *Transklik* reactie: TAD reageert in eerste reactie met een 1*H*-indool. Na opwarmen van het adduct, in het bijzijn van een dieen, wordt de TAD molecule overgezet naar het DA adduct. Hierbij wordt ook het start indool terug bekomen. Elke reactie die hierin beschreven wordt, voldoet aan alle eigenschappen van een *klikreactie*.

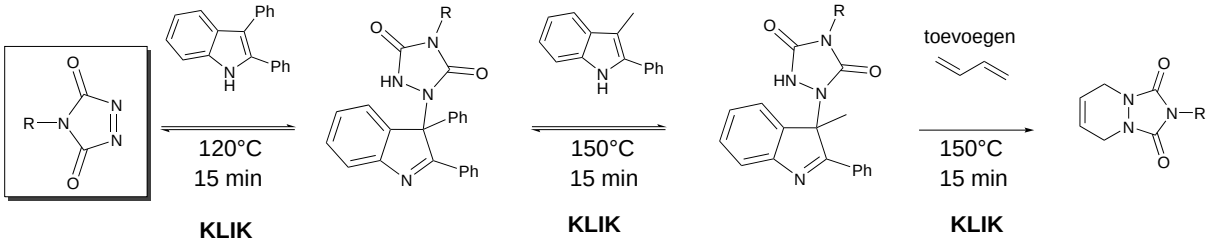
Dit *transklik* concept werd aangetoond op macromoleculair niveau door het eerst synthetiseren van een blockcopolymeer (gebaseerd op TAD-indool chemie) die in een volgende step werd verwarmd - in het bijzijn van een chemisch 'vangnet' (een dieen) - waarbij één van de originele polymeren werd vrijgesteld en de andere covalent gebonden werd met een andere reactie partner (zie Figuur IX.7). In een laatste stap, werden polymeernetwerken geproduceerd, op basis van TAD-indool chemie, die dynamisch gedrag vertonen. Deze netwerken toonden interessante eigenschappen zoals recyleerbaarheid, herwerkbaarheid...



Figuur IX.7: *Transklik* reactie met polymeren: een TAD-gefunctionaliseerd polymeer reageert in een eerste *klikreactie* met een 1*H*-indool-gefunctionaliseerd polymeer. Na verwarmen van het blockcopolymeer, in bijzijn van een dieen, wordt het TAD-polymeer getransfereerd naar het DA adduct en wordt het originele indool-polymeer vrijgesteld.

Geïnspireerd door het *aanpasbaar kinetisch gedrag* van TAD reacties, ontstond de vraag of de reversibele TAD-indool reactie ook nog verder aangepast kon worden. In hoofdstuk VII, werden verschillende TAD-indool combinaties bestudeerd, waarbij sommige een significant lagere reversibiliteitstemperatuur vertoonden (tot 20 °C). Hierna

werd het nieuw geïntroduceerde *transklik* concept uitgebreid met deze TAD-indool combinatie. Dit leidde tot het ontwikkelen en implementeren van een *opeenvolging van drie verschillende klikreacties*. Hierbij werd een TAD molecule selectief getransfereerd van een eerste TAD-indool adduct (gebaseerd op 2,3-fenyl-1*H*-indool) naar een tweede TAD-indool combinatie (gebaseerd op 2-fenyl-3-methyl-1*H*-indool) en in een laatste stap naar het DA adduct (zie Figuur IX.8). Voor zover wij weten, is dit het eerste voorbeeld van zo een selectieve en zuivere transfer via *klikreacties*. De volgende stap hield in dit opnieuw te bewijzen op polymeermaterialen, maar door gebrek aan tijd is dit jammer genoeg niet meer vervat kunnen worden in dit werk.



Figuur IX.8: 'Indool-indool-dieen'*transklik* reactie: een vereenvoudigde voorstelling van de *kliktransfer* van een TAD molecule op drie niveaus.

In dit doctoraatsonderzoek werd de basis van een nieuw ontwikkeld *klikchemie* platform, gebaseerd op de unieke reactiviteit van triazolinedionen, beschreven. Behalve het feit dat TAD-chemie kan aanzien worden als nog een extra *klikchemie*, bezit het ook nog een uitzonderlijk kinetisch gedrag dat moduleerbaar is (voor de snelheidsconstanten kan een verschil van 8 grootte-ordes bekomen worden). Anderzijds kunnen reversibele verbindingen ingevoerd worden via TAD-indool chemie. In functie van dit laatste werd een nieuw concept (een *transklikreactie*) geïntroduceerd, wat een uniek 'transfer' gedrag van TAD-moleculen beschrijft.

IX.4 Vooruitblik

Tijdens de loop van een doctoraatsonderzoek komen constant nieuwe ideeën en projecten naar boven. Dit werk is daarop geen uitzondering. Zoals reeds uitvoerig beschreven, zijn er verschillende nieuwe doctoraatsprojecten (zes binnen de eigen onderzoeksgroep en twee industriële) opgestart tijdens dit vierjarige onderzoek. Daarbij komen nog een twintigtal samenwerkingen met (inter)nationale onderzoeksgroepen in verscheidene onderzoeksgebieden. Ondanks het veelvuldig onderzoek dat op dit ogenblik gaande is, blijven er nog belangrijke onderwerpen die, in de nabije toekomst, bestudeerd moeten worden.

Door het beperkte aanbod aan commercieel beschikbare TAD componenten (zie hoofdstuk III), blijft er nog steeds een grote nood aan het synthetiseren van deze moleculen. Dit onderzoek heeft gepoogd om hiervoor een startpunt te zijn door onderzoek te verrichten naar algemene en opschaalbare syntheseroutes voor TAD-moleculen. Voor vernetters en niet-functionele molecules was dit een succes, maar er blijft nog steeds ruimte voor betere syntheses die leiden tot functionele TAD-bouwstenen. Alhoewel sommige van deze TAD-componenten reeds gemaakt werden, bleef dit altijd op kleine schaal en beperkt tot zeer specifieke toepassingen. Naar onze mening zal het brede publiek TAD-chemie pas omarmen als verder onderzoek verricht wordt naar algemene syntheseroutes.

Eens meer functionele TAD-moleculen beschikbaar zijn, kan de stap gezet worden naar meer complexe applicaties (en de daaruit volgende materiaaleigenschappen). Bijvoorbeeld worden in de polymeerwetenschappen efficiënte (*klik*)reacties gebruikt voor de synthese van complexe architecturen (dendrimeren, hypervertakte polymeren,...) en cyclische polymeren. Door het ontwikkelen van deze structuren zal het *klikgedrag* van TAD chemie verder versterkt worden. Als extra voordeel is er het aanpasbaar gedrag (zowel kinetisch als thermodynamisch) van TAD-reacties, wat kan leiden tot nieuwe inzichten en toepassingen. Het nieuw geïntroduceerde *transklik* concept met zijn programmeerbare en uiterst betrouwbare transfer van TAD reagentia (tussen drie verschillende substraten) opent nieuwe mogelijkheden. Inderdaad, dit kan bekeken worden als een overdracht van chemicaliën vanuit één specifieke locatie (al dan niet met behulp van een modulator) naar andere, wat sterk doet denken aan chemische signalen die gewoonlijk alleen

waargenomen worden in celbiologische systemen, maar dan met covalente reactiviteits-gebaseerde interacties ipv supramoleculaire affiniteits-gebaseerde interacties.

Een laatste belangrijk aspect, dicht gerelateerd met de reversibiliteit, is de temperatuurslimiet van TAD-moleculen. Zoals besproken in sectie III.5.1 mogen deze structuren niet boven 150 °C verwarmd worden om dimerisatie te vermijden. Omdat dit een intrinsieke eigenschap is van een TAD-molecule, is dit een zeer belangrijke eigenschap om rekening mee te houden, zeker wanneer TAD-chemie wordt geïntroduceerd in een nieuw domein en/of applicatie. Zolang de temperatuur onder 150 °C blijft, zouden er geen problemen mogen ontstaan, maar dit beperkt het aantal mogelijke chemische processen natuurlijk wel in grote mate.

We kunnen besluiten dat er nog steeds veel uitdagingen zijn voor TAD-chemie, zeker op synthesevlak. Anderzijds zijn we er sterk van overtuigd dat de beperkingen van dit chemieplatform niet zullen opwegen tegen de vele voordelen (en mogelijkheden) en dat TAD-chemie snel zijn weg zal vinden binnen de chemische wereld.

IX.5 Bibliografie

[1] J. E. Moses, A. D. Moorhouse, *Chemical Society Reviews* **2007**, *36*, 1249–1262.

[2] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angewandte Chemie International Edition* **2001**, *40*, 2004–2021.

[3] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angewandte Chemie International Edition* **2002**, *114*, 2708–2711.

[4] C. W. Tornøe, C. Christensen, M. Meldal, *The Journal of Organic Chemistry* **2002**, *67*, 3057–3064.

[5] C. J. Hawker, V. V. Fokin, M. G. Finn, K. B. Sharpless, *Australian Journal of Chemistry* **2007**, *60*, 381–383.

[6] A. Inglis, S. Sinnwell, M. Stenzel, C. Barner-Kowollik, *Angewandte Chemie International Edition* **2009**, *48*, 2411–2414.

[7] C. Barner-Kowollik, F. E. Du Prez, P. Espeel, C. J. Hawker, T. Junkers, H. Schlaad, W. Van Camp, *Angewandte Chemie International Edition* **2011**, *50*, 60–62.

[8] W. Xi, T. F. Scott, C. J. Kloxin, C. N. Bowman, *Advanced Functional Materials* **2014**, *24*, 2572–2590.

[9] B. S. Sumerlin, A. P. Vogt, *Macromolecules* **2010**, *43*, 1–13.

[10] P. L. Golas, K. Matyjaszewski, *Chemical Society Reviews* **2010**, *39*, 1338–1354.

[11] T. Pauloehrl, G. Delaittre, V. Winkler, A. Welle, M. Bruns, H. G. Börner, A. M. Greiner, M. Bastmeyer, C. Barner-Kowollik, *Angewandte Chemie International Edition* **2012**, *51*, 1071–1074.

[12] M. A. Tasdelen, Y. Yagci, *Angewandte Chemie International Edition* **2013**, *52*, 5930–5938.

[13] R. C. Cookson, S. S. H. Gilani, I. D. R. Stevens, *Tetrahedron Letters* **1962**, *3*, 615–618.

[14] M. A. Tasdelen, *Polymer Chemistry* **2011**, *2*, 2133–2145.

[15] J.-A. Funel, S. Abele, *Angewandte Chemie International Edition* **2013**, *52*, 3822–3863.

[16] K. C. Nicolaou, S. A. Snyder, T. Montagnon, G. Vassilikogiannakis, *Angewandte Chemie International Edition* **2002**, *41*, 1668–1698.

[17] S. Radl in *1,2,4-Triazoline-3,5-Diones*, *Vol. 67*, Elsevier, San Diego, **1997**.

[18] I. K. Korobitsyna, A. V. Khalikova, L. L. Rodina, N. P. Shusherina, *Chemistry of Heterocyclic Compounds* **1983**, *19*, 117–136.

[19] C. Barbas, H. Ban, J. Gavrilyuk, *US20120289682* **2010**, Tyrosine Bioconjugation through Aqueous Ene–Like Reactions.

[20] G. B. Butler, *Industrial and Engineering Chemistry Product Research and Development.* **1980**, *19*, 512–528.

[21] K. De Bruycker, S. Billiet, H. Houck, S. Chattopadhyay, J. Winne, F. Du Prez, *Chemical Reviews* **2015**.

[22] M. L. Blackman, M. Royzen, J. M. Fox, *Journal of the American Chemical Society* **2008**, *130*, 13518–13519.

[23] S. Billiet, K. De Bruycker, F. Driessen, H. Goossens, V. Van Speybroeck, J. M. Winne, F. E. Du Prez, *Nature Chemistry* **2014**, *6*, 815–821.

[24] O. Türünç, S. Billiet, K. De Bruycker, S. Ouardad, J. Winne, F. E. Du Prez, *European Polymer Journal* **2015**, *65*, 286–297.

Appendix A

List of publications

Peer-reviewed articles

Billiet S., De Bruycker K., Driessen F., Goossens H., Van Speybroeck V., Winne J. M., Du Prez F. E.,*Nature Chemistry*, **2014**, *6* (9), 815.
Triazolinediones enable ultrafast and reversible click chemistry for the design of dynamic polymer systems.

Türünç O., Billiet S., De Bruycker K., Ouardad S., Winne J.M., Du Prez F.E., *European Polymer Journal* **2015**, *65*, 286.
From plant oils to plant foils: straightforward functionalization and crosslinking of natural plant oils with triazolinediones.

De Bruycker K., Billiet S., Houck H.A., Chattopadhyay S., Winne J.M., Du Prez F.E., *Chemical Reviews*, **2015**, submitted.
Triazolinediones as highly enabling synthetic tools.

Billiet S., Houck H.A., De Bruycker K., Winne J.M., Du Prez F.E., *to be submitted.*
Transclick reaction: towards a controlled cascade of click *reactions.*

Patent application

Billiet S., De Bruycker K., Winne J.M., Du Prez F.E., **WO 2015/018928 A1**
Urazole compounds

Peer-reviewed articles outside this doctoral work

Billiet S., Van Camp W., Hillewaere X.K.D., Rahier H., Du Prez F.E., *Polymer*, **2012**, *53*, 2320.
Development of Optimized Autonomous Self-Healing Systems for Epoxy Materials Based on Maleimide Chemistry.

Billiet S., Hillewaere X.K.D., Texeira R.F.A., Du Prez F.E., *Macromolecular Rapid Communications*, **2013**, *34*, 290.
Chemistry of Crosslinking Processes for Self-Healing Polymers

Wang Z., Yuan W., Trenor N.M., Vlaminck L., Billiet S., Sarkar A., Du Prez F.E., Stefik M., Tang C., *Green Chemistry*, **2015**, *17*, 3806.
Sustainable thermoplastic elastomers derived from plant oil and their "click-coupling" via TAD chemistry

Wang Z., Zhang Y., Yuan L., Hayat J., Trenor N.M., Vlaminck L., Billiet S., Du Prez F.E., Wang Z., Tang C., *submitted*
A Biomass Approach toward Robust, Sustainable Multiple Shape-Memory Materials

Book chapter

Texeira R.F.A, Hillewaere X.K.D., Billiet S., Du Prez F.E., *Wiley*, **2013**
Self-Healing Polymers: From Principles to Applications (Chapter 9: Chemistry of Crosslinking Processes for Self-Healing Polymers)